FEBS Letters 589 (2015) 1669–1677

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Growth control mechanisms in neuronal regeneration

Ella Doron-Mandel, Mike Fainzilber, Marco Terenzio *

Department of Biological Chemistry, Weizmann Institute of Science, 76100 Rehovot, Israel

ARTICLE INFO

Article history:
Received 29 March 2015
Revised 20 April 2015
Accepted 21 April 2015
Available online 1 May 2015

Edited by Wilhelm Just

Keywords:
Nerve injury
Central nervous system
Peripheral nervous system
Axonal transport
Axonal mRNA translation
Regeneration

ABSTRACT

Neurons grow during development and extend long axons to make contact with their targets with the help of an intrinsic program of axonal growth as well as a range of extrinsic cues and a permissive *milieu*. Injury events in adulthood induce some neuron types to revert to a regenerative state in the peripheral nervous system (PNS). Neurons from the central nervous system (CNS), however, reveal a much lower capacity for regenerative growth. A number of intrinsic regeneration-promoting mechanisms have been described, including priming by calcium waves, epigenetic modifications, local mRNA translation, and dynein-driven retrograde transport of transcription factors (TFs) or signaling complexes that lead to TF activation and nuclear translocation. Differences in the availability or recruitment of these mechanisms may partially explain the limited response of CNS neurons to injury.

1. Introduction

Neurons extend their axons over great distances during development to make contact with their targets. This is achieved with the help of many signaling pathways and within a growth-favoring *milieu*. However, after the establishment of these contacts, such intrinsic capacity is greatly reduced or lost, especially in the central nervous system (CNS) [1–3]. Often, following a traumatic event, there is the need for neurons to regenerate and revert back to an "elongation mode" that characterizes the developmental stage. While regeneration occurs in the peripheral nervous system (PNS), adult CNS neurons have a vastly reduced regeneration capacity [4]. This disparity underlies the interest in understanding the differences between these two systems in order to discover pathways that facilitate axonal regeneration.

One of the major differences between the CNS and PNS is the surrounding environment in which the injured axons try to regenerate. In the CNS many factors derived from various supporting cells, including myelinating oligodendrocytes, contribute to the creation of a growth-inhibitory environment after injury either by forming physical barriers or, alternatively, by receptor mediated repulsion (reviewed in [5,6]). The most prominent of the latter are the extra-cellular domain of the protein Nogo-A, myelin-associated glycoprotein (MAG) and oligodendrocyte/myelin glycoprotein (OMgp), ephrin B3, ephrin A3, semaphorin 4D, semaphorin 5A, semaphorin 3F, as well as chondroitin sulfate proteoglycans (CSPGs) and the myelin glycolipid sulfatide [6]. In addition, in the CNS a 'glial scar' is formed upon injury by migration of astrocytes, proliferation of reactive astrocytes and accumulation of intermediate filament proteins such as the glial fibrillary acidic protein (GFAP), vimentin and others [6]. A very recent paper showed that systemic administration of a blood–brain barrier permeable microtubule stabilizing drug, epothilone B (epoB), was able to decrease the extent of scarring after spinal cord injury in rodents by interfering with the migration of scar-forming fibroblasts. This drug was also able to induce microtubule polymerization in the axon tip, consequently promoting axonal growth and regeneration, resulting in an improved motor function after the lesion [7].

All of the aforementioned processes are regarded as "extrinsic" cues that prevent CNS regeneration. In accordance with these observations, it was shown that CNS neurons are able to regenerate their axons when given a growth-permissive substrate such as a peripheral nerve graft [8] or a stem-cell derived *milieu* [9], although regeneration beyond these environments is usually very limited. However, a favorable environment is not sufficient for efficient regeneration in CNS neurons [10] and manipulations that successfully enhance axonal regeneration often require combinations of factors affecting both extrinsic and intrinsic mechanisms [11], suggesting that CNS neurons may lack intrinsic mechanisms for promotion of a "regenerative state". The ability of PNS axons to regenerate after injury presents an opportunity to study the

* Corresponding author.
E-mail address: marco.terenzio@weizmann.ac.il (M. Terenzio).

http://dx.doi.org/10.1016/j.febslet.2015.04.046

intrinsic mechanisms underlying axonal regeneration. In this review we will explore the nature of the intrinsic and extrinsic mechanisms that lead to regeneration by comparing the differences between CNS and PNS neurons.

2. The conditioning lesion paradigm

One of the most common models that exemplify the intrinsic differences between the regenerative potential of CNS and PNS neurons is the so-called "conditioning lesion" paradigm. Dorsal root ganglia (DRG) sensory neurons are characterized by a bifurcating axon with two branches, a peripheral and a central branch. While the peripheral branch has the ability to regenerate following injury, the central process does not. Interestingly, if the peripheral branch is injured prior to the central tract lesion, centrally projecting neurites regain their ability to regenerate *in vivo* [12–14]. The "conditioning lesion effect" is observed in DRG neurons grown in culture after injury of their peripheral branch, whereby they shift from their normal highly-branched "arborizing" morphology to an elongating modality of growth [14,15]. DRG neurons cannot, however, be similarly conditioned by an injury in the central branch [15], unless the central lesion was preceded by a peripheral injury [13]. This evidence strongly suggests that, while peripheral branch injury can increase the intrinsic growth capacity of DRG neurons, the same is not true for injury of the central branch.

Findings from the conditioning lesion model raise the question: what are the molecular basis of a regenerative response that can be mounted as a consequence of a peripheral lesion, but not a central one, in the exact same cell? In the following sections of this review we will focus on several different mechanisms that coordinate the regenerative response, including calcium waves, epigenetic modifications, active retrograde macromolecular transport, transcriptional response and local protein synthesis. For each one of these mechanisms we will discuss what is known in terms of differences between PNS and CNS neurons.

3. Calcium waves

Calcium influx in the axoplasm represents a fast signaling avenue in response to injury that is able to trigger several mechanisms connected to axonal growth. The consequent inversion of the normal calcium/sodium flux creates a depolarization that is propagated along the axon all the way to the cell body [16]. In *Caenorhabditis elegans* the amplitude of such depolarization waves may correlate positively with the extent of sensory neuron regeneration, while the opposite is also true, in that inhibition of calcium signaling reduces the regenerative potential of injured axons [17]. Interestingly, the underlying mechanism inducing a calcium wave in response to injury between central and peripheral neurons might differ greatly. For instance, while it was shown that in cortical neurons the generation of a calcium wave requires both calcium and sodium voltage-dependent channels [18], in sympathetic neurons there is no such dependency on sodium channels [19].

Changes in intracellular calcium levels activate downstream effectors that in turn regulate regeneration. One of the most prominent calcium responsive mechanisms is the activation of adenylate cyclase (AC) and the subsequent raise in cyclic AMP (cAMP) levels. Indeed, local increase of cAMP influences the establishment of a functional growth cone after axotomy. In *C. elegans* sensory neurons calcium-dependent enzymes lead to an increase of cAMP, which promotes the rearrangement of the cytoskeleton needed for the growth cone assembly [17]. In rodents, activation of extracellular-signal regulated kinase 1,2 (ERK) is required for the formation of a competent growth cone after axotomy in dorsal root ganglia axons, while depletion of extracellular calcium or the inhibition of cAMP – protein kinase A (PKA) significantly impair this process [20]. Similar results have also been reported in *Aplysia*, where the assembly of an effective growth cone machinery, which is able to initiate axonal regeneration, is dependent on calcium influx [21]. Indeed, structural organization of the cytoskeleton at an axonal lesion site is altered by conditions that limit calcium influx [21]. Such alterations cause delays in the fusion of anterogradely transported vesicles to the plasma membrane of the cut axonal end, affecting the ability of the growth cone to regenerate [21]. Along the same lines, cAMP promotes regeneration in peripheral sensory neurons, where its levels are elevated after injury [22].

It is not clear, however, whether cAMP can contribute to CNS neurons regeneration. The effect of cAMP on the regeneration of sensory neurons after spinal cord lesion was modest in comparison to its effects after peripheral injury in the same cell type [23]. In retinal ganglia cells (RGCs) cAMP was suggested to facilitate cell survival rather than axonal regeneration [24], and to play a role in modulating inflammation-induced regeneration, through its effects on oncomodulin binding in the retina [25].

A recent study has determined that the release of calcium from internal stores is essential to the generation of a calcium wave after nerve injury of mouse sensory neurons (Fig. 1A) [26]. Furthermore, the back-propagating calcium wave following axonal injury in DRG neurons arrives all the way to the soma and causes nuclear export of the histone deacetylase 5 (HDAC5) in a protein kinase Cμ (PKCμ) – dependent manner (Fig. 1A). This event facilitates axon regeneration *in vitro* and *in vivo* by leading to an increased acetylation of histone H3, thus inducing the up-regulation of regeneration-associated genes (RAGs) such as Jun, KLF4, KLF5, Fos, and Gadd45a [26] (Fig. 1A). An interesting suggestion is that this early calcium-dependent mechanism of HDAC5 nuclear export primes the neuronal cell body for a second slower signaling dependent on retrograde transport along microtubules in the axon [26] (see below). In addition, following its injury-induced nuclear export, HDAC5 is transported to axon tips where it accumulates, ultimately resulting in local deacetylation of tubulin, which in turn promotes growth-cone dynamics and axon regeneration [27]. Interestingly, the aforementioned PKCμ activation and increased histone acetylation was not observed in RGCs; and HDAC5 accumulation and consequent tubulin deacetylation was not detected in the axon tips of RGCs [26,27]. These observations support the notion that one or more of the steps in the cascade of events stemming from the back-propagating calcium wave to the activation of PKCμ and nuclear export of HDAC5 differ between CNS and PNS neurons. The exact steps underlying the disparity between the two systems remain, however, to be determined.

A key difference between the central and the peripheral response to axonal injury is the tendency of central axons to form a retraction bulb, while peripheral axons form a growth cone that enables regeneration a short time after injury [28]. This differential response seems to be mediated by the dynamics of microtubules leading to stabilization (growth cone) in the case of the PNS and de-stabilization (retraction bulb) in the case of the CNS [29]. In *Aplysia* neurons, axonal lesion was shown to cause a re-orientation of microtubule polarity at the cut end, which supports the sorting and concentrating of different membrane resources to specific sites on the injured axon, thereby transforming the axonal stump into a motile growth cone [30]. While a detailed overview of this topic is outside the scope of the review, we will mention that the major identified effectors of this differential response are the histone deacetylases HDAC6 [31], HDAC5 [27] and the kinesin family member KIF3C [32]. Specifically, axonal injury triggers microtubule deacetylation in PNS but not in CNS neurons [27]. Histone deacetylation has been suggested to be connected with microtubule dynamics, which are essential for the

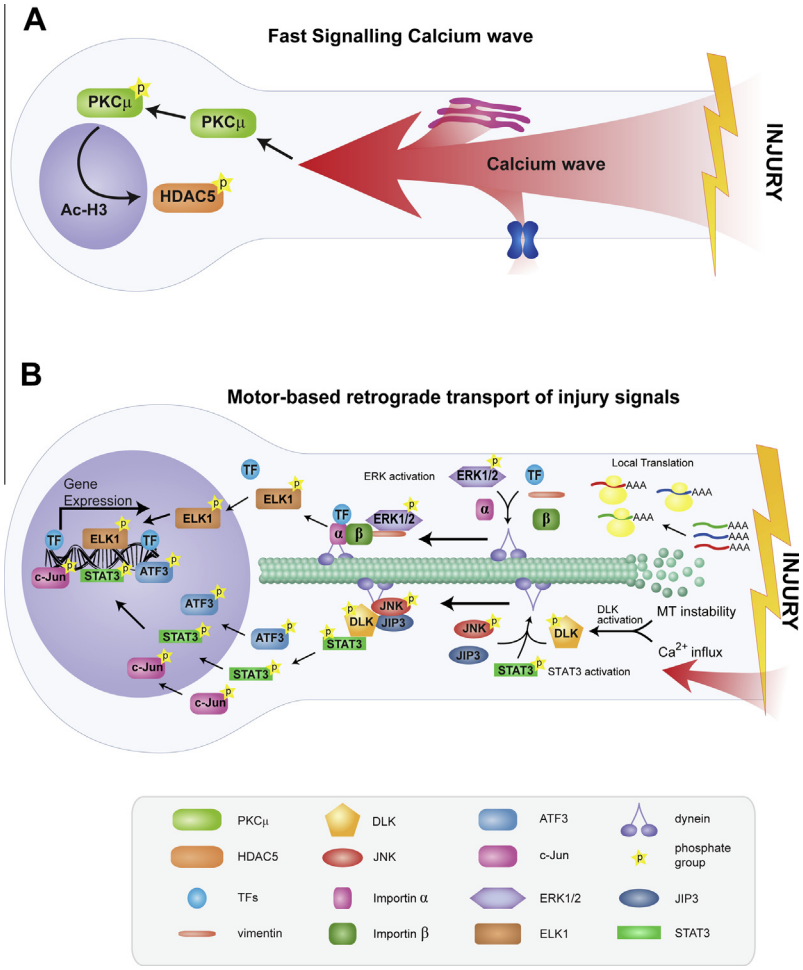

Fig. 1. Mechanisms of response to injury in PNS neurons. The schematic depicts early and late events in response to axonal injury in PNS neurons. An initial fast calcium wave is initiated at the injury site as a consequence of the rupture of the axonal membrane and the opening of sodium/calcium channels. The resulting increase in calcium levels in the axons is likely reinforced by calcium release from intracellular stores such as the endoplasmic reticulum. The ionic wave is then propagated all the way to the cell soma, where it can elicit the nuclear export of histone deacetylase 5 (HDAC5), which in turn primes the chromatin for transcription via acetylation (Ac) of the histone 3 (H3). B. A second slower signaling wave, which is dependent on dynein retrograde transport, is sent to the cell soma. Axonal injury is able to activate local translation in PNS neurons. Importin β1 and vimentin are translated among other mRNAs. The first, due to its association with importin α and NLS-bearing transcription factors (TFs), leads to the formation of a retrograde injury signaling complex, associated by an importin α to dynein. In addition, accessory binding sites for vimentin enable dynein-mediated transport of phosphorylated extracellular signal-regulated kinases 1/2 (ERK1/2). Upon arrival in the soma, individual components of this signaling complex can activate downstream signals such as ETS domain-containing protein 1 Elk-1 (ELK1) or directly translocate to the nucleus. The signal transducer and activator of transcription 3 (STAT3) is also activated following injury and is transported to the nucleus due to its interaction with impotin α. The dual leucine zipper kinase (DLK), which is activated locally after injury, can also mediate STA3 transport to the cell soma. Finally, Jun amino-terminal kinase (JNK) local activation leads to its loading onto dynein in concert with Sunday Driver, also known as JIP3, and DLK. This signaling complex activates c-Jun and after arriving to the cell soma leads to the activation of the activating transcription factor 3 (ATF3). The TFs translocated into the nucleus ultimately contribute to the induction of a transcriptional response that leads to the activation of a regeneration program.

formation of a regeneration competent growth cone [27]. Indeed, HDAC5 knockdown was shown to reduce microtubule dynamics, while its overexpression had the opposite effect [27]. Likewise, KIF3C localization at the tip of growing microtubules is necessary for the maintenance of the growth cone's dynamic behavior and its depletion was shown to impair regeneration after injury in DRG neurons both *in vitro* and *in vivo* [32]. Further study of these molecules is ongoing and their potential involvement in facilitating axonal regeneration in the CNS will be an interesting topic for further studies [28].

4. Epigenetic modifications

Epigenetic modifications constitute another regulatory mechanism that can govern neuronal growth states. In addition to the aforementioned work of Cho et al. (2013), the importance of histone acetylation in axonal regeneration had been inferred by the observation that valproic acid, a histone deacetylase inhibitor, improves motor recovery after spinal cord injury in rat [33]. The connection between epigenetic regulation and response to injury was further strengthened by the observation that histone 4 acetylation is restored following axonal injury, while histone-modifying enzymes were found to act together with the transcription factor Smad1 to facilitate transcriptional regulation of RAGs [34]. Interestingly, a recent paper demonstrated a role for the Smad1 pathway in promoting axon regeneration in the mammalian nervous system through a mechanism that is dependent upon glycogen synthase kinase 3 (GSK3) – mediation of PI3K-dependent signaling [35]. In addition, depletion of Smad1 in adult mice was shown to prevent axon regeneration *in vivo* [35]. Several other epigenetic mechanisms have been described in the context of axonal regeneration. Notably, the transcriptional complex formed by the tumor suppressor p53 and its acetyltransferases CBP/p300, which results in the acetylation of histone 3, has been reported to promote the initiation of a regeneration program involving RAGs, such as for example the axonal growth-associated protein 43 (GAP-43)

[36–38]. Finally, further *in vivo* evidence of the importance of this mechanism was given by the observation that in an optic nerve crush model of axonal injury, adenoviral-mediated overexpression of p300 promotes axonal regeneration in the optic nerve [39].

Another epigenetic modifier that was suggested to contribute to successful regeneration is the histone acetyltransferase p300/CBP-associated factor (PCAF), which regulates RAG expression by acetylation of their associated histone 3 [40]. Importantly, PCAF dependent gene reprogramming of RAGs only occurs after peripheral, rather than central, injury in DRG neurons and it is necessary for the conditioning lesion effect *in vitro* [40]. Interestingly, it seems that NGF-activated ERK retrograde injury signaling is needed for PCAF's effect on gene expression [40]. The observation that ERK retrograde signaling is needed in order to enable PCAF dependent epigenetic changes to induce regeneration suggests a direct link between retrograde injury signaling to epigenetics of RAGs.

5. Transport of locally activated injury signals

We previously discussed the possible role of a fast back-propagating calcium wave as a first line of response to injury; the contribution of a second slower signaling wave mediated by molecular motors has also been extensively studied. In regards to injury signals, both anterograde and retrograde microtubule-based transport are important for the regeneration response. While discussing the contribution of the anterograde transport in injury regeneration is outside the scope of this review, we will explore the retrograde dynein-dependent injury signaling.

Aplysia was widely used in the early work on injury signaling due to its large neurons. Taking advantage of this feature, injections of axoplasm from crushed but not from uncrushed nerves into the soma of uninjured sensory neurons resulted in the triggering of an injury-like response in these cells. These observations suggested that axonal injury activates molecular signals locally in the axon [41]. Since the signals activated locally in the axoplasm must be transported to the nucleus, the possibility was explored that at least some of them could be proteins containing a nuclear localization signal (NLS) [41,42]. In rodents this mechanism was later confirmed by the observation that the introduction of synthetic NLS peptide at the injury site of axotomized sensory neurons or injection of NLS directly in the sciatic nerve was able to impair the regenerative response of these cells [43].

Importin α and β are major components of the nuclear import complex. NLS-containing proteins exhibit a low binding affinity to importin α and high binding affinity to α/β heterodimers. While multiple importin α isoforms are present as protein in intact nerves, that is not the case for importin β1, which needs to be locally translated at the injury site [43] (Fig. 1B). Importantly, Ras-related nuclear protein binding protein 1 (RanBP1) is also locally translated after injury, triggering the hydrolysis of the resident RanGTP, which detaches from importin α, thus freeing the latter to interact with the newly synthesized importin β1 to form the retrograde nuclear import complex [44]. Indeed, perturbing the hydrolysis of RanGTP or blocking RanBP1 at axonal injury sites attenuates the neuronal conditioning lesion response [44].

In rodent DRG neurons, another signaling molecule locally activated after injury and involved in the retrograde signaling that initiates regeneration is ERK [45] (Fig. 1B). This protein is transported retrogradely to the neuronal cell body by the same transport machinery by virtue of its association with locally synthesized vimentin [45] (Fig. 1B), which binds phosphorylated ERK 1/2, thus linking them to dynein via its direct binding to importin β1 [45] (Fig. 1B). Other retrograde injury signaling molecules can bind to dynein in an importin-independent manner. Specifically, nerve injury induces the local activation of c-Jun N-terminal kinase (JNK), which is then transported retrogradely by virtue of its association with dynein [46] (Fig. 1B). Sunday Driver (syd), also known as JIP3 was identified as a motor adaptor for axonal endosomes [47]. In response to axonal injury, syd mediates the retrograde transport of JNK-positive vesicles, which constitute a mobile signaling platform to relay axonal injury signal to the cell body [47] (Fig. 1B). Importantly, JNK has been implicated in the re-organization of the neuronal cytoskeleton through the microtubule-associated protein 1B (MAP1B) and its signaling was shown to be required for sustained neurite elongation, as JNK inhibition results in neurite retraction [48]. It should be noted though, that there are several JNK isoforms and they seem to have distinct roles in regards to neurite elongation and regeneration [48].

6. Transcriptional response to injury

In order to successfully regenerate, neurons must mount an injury response by changing their gene expression [49]. A number of Regeneration Associated Genes (RAGs) have been identified including growth-associated protein 43 (GAP-43), the cortical cytoskeleton-associated protein of approximate molecular mass 23kDa (CAP-23), the small proline-rich protein 1A (SPRR1A) and various cytoskeletal components (reviewed in [14]). The expression of RAGs is coordinated by transcription factors associated with the regeneration program, such as c-Jun [50], SRY-box containing gene 11 (Sox-11), [51] and the activating transcription factor 3 (ATF3) [52]. Indeed, ATF3 activation following peripheral injury in DRG neurons leads to increased expression of SPRR1A [52], and is able to promote neurite outgrowth by modulating the intrinsic growth capacity of these cells [53]. Crucially, in DRG neurons ATF3 upregulation only happens if the injury is located in the peripheral axonal branch, but not in the central one [54].

The previously mentioned role for the nuclear importin complex in injury-dependent retrograde transport suggests a possible direct involvement of transcription factors (TFs), which are normally translocated to the nucleus by virtue of their NLS sequence, in the signaling triggered by axonal injury. The Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway is a major modulator of growth. Signal transducer and activator of transcription 3 (STAT3) in particular was shown in many studies to be a potent inductor of cell survival and regeneration [55–57]. Indeed, STAT3 was shown to be locally translated and activated upon injury, and transported via its association with dynein and importin α5 to DRG nuclei, where it modulates the survival of these neurons [56]. Interestingly, a recent paper reported that the dual leucine zipper kinase (DLK), a mitogen-activated protein kinase kinase kinase (MAPKKK) that can activate JNK, is necessary for retrograde transport of activated p-STAT3 to the cell body after sciatic nerve injury [58] (Fig. 1B). DLK can be activated also by the disruption of actin or microtubule cytoskeleton in a manner that is independent from calcium influx and this activation induces a pro-regenerative state akin to preconditioning, which enhances the response to injury [59] (Fig. 1B). Previously discussed signaling molecules, which are triggered as a consequence of injury, such as ERK and JNK, can also activate transcription factors. Indeed, ERK triggers the activation of the ETS domain-containing protein (Elk-1) [45] (Fig. 1B). On the other hand, JNK activates ATF3 as demonstrated by the observation that inhibition of axonal transport or axonal inhibition of JNK activation reduces the number of ATF3-positive neuronal nuclei [60] (Fig. 1B). Other TFs have been implicated in regeneration such as the cAMP response element-binding protein (CREB), and Smad1 [15,35,61,62]. A recent study highlighted the importance of AP1, another TF suggested to be activated by cAMP [63]. Its activation in conjunction with CREB activation was shown to be necessary to stimulate the

gene expression that leads to enhanced axonal regeneration [63]. A more comprehensive overview of the transcription factors involved in injury response can be found in Tedeschi (2012) [49].

7. Local protein synthesis

An increasing number of studies have recently emphasized the role of axonal local translation of proteins after injury. The ability of axons to locally synthesize proteins, distant from the cell body, was proposed to be important for their capacity to regenerate. This hypothesis was based on the identification of several mRNAs localized within axons and observations suggesting the presence of ribosomes along axons and growth cones [64]. Indeed, axonal processes of adult sensory neurons cultured after conditioning injury were shown to contain ribosomal proteins, translational initiation factors, and ribosomal RNA [64]. Growth cone's retraction and/or reduction in the number of regenerating growth cones was also observed from transected axons in presence or absence of inhibitors of protein synthesis and after the communication with the cell body was compromised by axotomy or inhibition of axonal transport [64,65]. Similar results could be observed following the inhibition of mTOR, p38 MAPK, and Caspase3, all key players in protein synthesis and degradation [65].

Interestingly, a connection between age and regenerative potential has also been identified, whereby axons of DRGs from younger mice have higher levels of the translational machinery and a higher capacity to regenerate after injury [20]. In addition, the use of compartmentalized filter cultures, which allows the isolation of pure axonal preparations, and genome-wide microarray technology, has shown that embryonic and adult DRG axons contain a largely overlapping repertoire of mRNAs with over 1100 transcripts unique to embryonic and over 1400 to adult axons [66]. Interestingly, embryonic axons were enriched in mRNAs with a role in axonal guidance and growth, regenerating and pre-lesioned adult axons in RNAs with a role in inflammation and immunity [66].

The presence of mRNA transcripts in axons suggests the existence of a mechanism by which these molecules are transported there in the first place. Axonal localization can be achieved by the binding of a specific mRNA with an RNA-binding protein. One of the most studied cases of such an interaction is the mRNA for β-actin, which contains a sequence for axonal targeting of 54-nucleotides within its 3′ untranslated region (UTR) called *zipcode*. The insertion of this sequence grants an mRNA the ability to bind to a protein called ZBP-1 (RNA-binding protein zipcode-binding protein 1), which is then responsible for its axonal localization [67,68]. Indeed, tagging the mRNA of myristilated green fluorescent protein (myrGFP) with the sequence of β-actin's 3′-UTR was shown to drive its axonal localization in both PNS and CNS neurons *in vivo* [69].

In accordance with the previously discussed observations, mice with reduced levels of ZBP-1 display an impaired regeneration response after sciatic nerve injury [70], while overexpression of the 3′-UTR region of β-actin is able to outcompete other mRNAs, such as GAP-43, for their binding to ZBP-1 [70], and the 3′-UTR of GAP-43 mRNA can deplete axons of endogenous β-actin mRNA [71]. It is well documented that the subcellular localization of mRNAs for both β-actin and GAP-43 is crucial for axonal growth. *In ovo* electroporation experiments with axonally targeted β-actin and GAP-43 mRNA have shown that axonal translation of β-actin mRNA supports axon branching while axonal translation of GAP-43 mRNA supports elongating growth of sensory axons into the chick spinal chord [71] and improves axonal regeneration after injury in a Spry2 knockout mice [38].

Another interesting example of an mRNA that is localized in axons and is important for axonal regeneration is importin β1. As previously discussed, importins are found in axons at great distances from the cell body. Importin β1 mRNA, in particular, is localized in axons of DRG neurons and is translated upon sciatic nerve injury [43] (Fig. 1B). As mentioned above, this process is of critical importance for the formation of α/β complexes and the mounting of a proper NLS-mediated injury response. Notably, axonal localization of importin β1 mRNA is encoded by its 3′-UTR [72] and mice lacking the axon-localizing region of importin β1 3′-UTR have lower level of importin β1 transcript in DRG axons and an attenuated regeneration response following sciatic nerve crush [72].

8. Growth suppression programs

The evidence described above suggests that limited regeneration in CNS neurons might stem in part from an inability to activate intrinsic growth pathways. In this context, growth-suppression programs might also play a major role in the diminished regeneration capabilities of CNS neurons. It is generally accepted that neurons gradually lose their regenerative capabilities during embryonic development [1]. This is especially true in the case of CNS neurons, where the activity of the mammalian target of rapamycin (mTOR) declines with the degree of their maturation [73,74]. This observation suggests that re-activation of mTOR in neurons could contribute in reverting these cells to a more immature and regeneration-prone state. Indeed, several studies have explored mTOR's ability to promote neuronal growth and regeneration, as detailed below.

mTOR is a serine/threonine protein kinase that acts in a complex with other proteins to regulate cell growth, proliferation, metabolism, motility and survival [75]. It was found that deletion of the phosphatase and tensin homologue (PTEN), a regulator of mTOR, significantly increases both neuronal survival and axonal regeneration following injury in retinal ganglion cells (RGCs) in an mTOR-dependent manner, since administration of rapamycin neutralizes these survival and regeneration effects [73]. PTEN is a lipid phosphatase that preferentially dephosphorylates phosphoinositide substrates. It negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate (PIP3) thus regulating mTOR activity via Akt signaling [76,77]. PTEN was previously implicated as a key growth regulator in neuronal cells, where it was shown to affect cell soma size in a PI3K and mTOR dependent manner [78]. Specifically, a transport mechanism involving the interaction between myosin Va (highly enriched in the nervous system) and PTEN has been described [78]. This interaction, thought to control PI3K signaling and neuronal size, depends on the phosphorylation of PTEN C-terminal domain and is antagonized via GSK3 and CK2 inhibition, indicating that these kinases might regulate neuronal soma size upstream of PTEN transport [78]. Another mTOR regulator implicated in axonal regeneration is the tuberous sclerosis protein 2 (TSC2), whose deletion is also able to promote regeneration in DRG neurons [79]. The TSC1/TSC2 heterodimer is an upstream inhibitor of mTOR that is inhibited by phosphorylation by Akt.

mTORC1 stimulates protein translation via two main downstream effectors: by inhibition of the eukaryotic translation initiation factor 4E-binding protein (4E-BP) and activation of the ribosomal protein S6 kinase 1 (S6K1). Indeed, it was found that S6K1 has a dual role in promoting CNS axon regeneration: while its activation is sufficient to promote axon regeneration, it is also able to inhibit PTEN deletion-induced axon regeneration through the feedback inhibition of PI3K pathway, which normally counteracts PTEN [80]. In addition, while 4E-BP inactivation promotes cell survival in RGCs, it is not sufficient in itself for axonal regeneration. Rather, it is necessary for promotion of axonal growth that stems from PTEN deletion [80]. A recent paper documented that survival

rates following axotomy vary dramatically among subtypes of RGCs, with alpha-RGCs (αRGCs) surviving preferentially [81]. The reason for this preferential survival was once again connected to the mTOR pathway as αRGCs have uniquely high levels of mTOR signaling and also selectively express osteopontin (OPN) [81], a receptor which is capable of stimulating mTOR activity [82]. Taken together, the evidence presented above support the concept that mTOR is a central player needed for overcoming intrinsic growth suppression and that its activity takes major part in regulating intrinsic regeneration in CNS neurons.

In contrast to the wealth of data regarding the role of mTOR in CNS neurons, its function in injured peripheral neurons is more controversial. Indeed, previous studies showed that while both activation of PI3K and inhibition of PTEN are able to promote neurite outgrowth in peripheral sensory neurons, this effect may be mTOR independent as it is not affected by treatment with rapamycin [83]. Instead, it seems that PI3K-dependent increase of growth potential in the PNS is a consequence of glycogen synthase kinase 3 (GSK3) inhibition by PI3K which, in turn, induces elevated expression of the transcription factor Smad1 [35]. Other studies, however, have shown that mTOR activity is upregulated in injured peripheral neurons and in turn induces the expression of the regeneration marker growth associated protein GAP-43 [79]. The same group was also able to show that syntaxin13 expression levels increase in injured nerves in an mTOR-dependent manner as a result of local protein synthesis and not axonal transport [84]. Furthermore, depletion of syntaxin13 in cultured DRG neurons impaired axon growth and regeneration [84]. Thus, further studies are needed in order to clarify the role of mTOR in peripheral nerve regeneration and determine whether mTOR activity and regulation differ between central and peripheral neurons.

Along the same lines and as previously discussed, another major modulator of intrinsic growth potential in CNS neurons is the JAK–STAT pathway. Interestingly, STAT3 levels were shown to increase as a result of peripheral, but not central, injury and inhibition of the JAK–STAT3 pathways abolished the conditional lesion effect [55–57]. It was also shown that lens injury, when conducted along with optic nerve crush, can greatly increase RGCs survival and regeneration [85] and that this correlates with elevated expression levels of RAGs [86]. It is thought that this effect is mediated by STAT cytokine-induced signaling occurring as a result of the inflammation process induced by the lens injury. Indeed, similarly to PTEN, suppressor of cytokine signaling 3 (SOCS3) deletion leads to increased regeneration in RGCs [87]. This effect is mediated by the cytokine receptor glycoprotein 130 (gp130), which is a regulator of the JAK-STAT pathway. In addition, it was recently shown that the krueppel like factor 4 (KLF4) is an inhibitor of cytokine induced STAT3 activity and that deletion of which enhances regeneration of RGCs in a STAT3 dependent manner [88]. Interestingly, combining SOCS3 and KLF4 deletions further enhances this effect [88]. In addition, deletion of PTEN and SOCS3 have a synergistic effect, which is significantly larger then the expected sum of their individual outputs [89]. S6K1 seems also to play a role, as deletion of SOCS3 concomitant with S6K1 activation yields increased regeneration similar to that observed by PTEN/SOCS3 double deletion [80]. The overall integrated mechanism for this additive effect remains to be elucidated.

9. Conclusions and future perspectives

The results summarized above reveal a multiplicity of communication mechanisms between axons and cell bodies after nerve injury. Clear understanding of the regulation and integration of such signals will be required for the development of new therapeutic approaches to peripheral and central nervous system injury and disease. Two major challenges currently hinder progress toward this goal, on one hand the multiplicity of injury/regenerative signals might reflect backup mechanisms ensuring the robustness of the system, on the other hand the likelihood of mimicking the full spectrum of responses to axonal injury by individual pharmaceuticals seems slim. Hence, despite significant advances in our understanding, therapeutic strategies for treating CNS injury are currently still limited.

Another way to tackle this issue is to perform large genomic and proteomic screening for system-wide identification of the relevant genes or proteins (e.g. [90,91]). Unfortunately to date, only a limited number of genes discovered by these screening approaches have been validated for function *in vivo*. Nevertheless, these studies have already yielded a general overview of the neuronal response to injury and allowed a systematic characterization of the molecular pathways involved. As a result, the complexity of the injury response is coming to light and it is becoming clear that regenerative growth also involves specific pathways that sense and respond to damage [91]. Indeed, while there is a plethora of common pathways, such as mTOR signaling, which are active both in developmental axonal growth and axonal regeneration, it seems that the latter cannot be viewed simply as a recapitulation of axonal elongation during development. The discovery of common pathways for both processes might however present an opportunity for future development. The identification of shared transcriptional or epigenetic nodes should also be further explored for future mimicking of the regenerative response of PNS neurons in the CNS.

Another very interesting challenge is posed by the understanding of the mechanisms underlining the way the previously described retrograde injury signals are recognized and integrated at the level of the cell body. For example, how do neurons keep track of where along the axons an injury has taken place? Several studies conducted in DRG neurons demonstrated a differential modulation of the transcriptional response to injury, which is dependent on the distance of the lesion from the cell body. For instance, the kinetics of JNK activation and ATF3 induction has been found to be dependent on the distance of the axotomy site from the DRGs [54,92]. Likewise, this distance determines the type and the extent of the response to injury in retinal ganglion cells, where there is a correlation between the extent of axonal regeneration and the distance between site of axotomy and cell body [93]. A number of hypotheses have been formulated in order to explain the diversity of cell body responses to differently located axonal lesions based on diffusion mechanisms, signaling waves or spatial gradients of protein abundance. While diffusion is not likely to function efficiently over long distances, the other mechanisms might also lack the spatial range required for utility in adult nerve injury [94–96].

Mathematical models for encoding the location and/or distance of a lesion site based on fundamental properties of motor-dependent transport have been proposed [97–99]. Simulations taking into account the velocity ranges for kinesin and dynein motor complexes supported primarily two models, one in which the cells measures the delay between the arrival of an early priming signal and that of a later motor-dependent signal [97], and one in which the spatial information is encoded by an oscillatory signal generated by bidirectional regulation of kinesin- and dynein-based signaling quanta [98]. From a computational point of view, the first of the two models requires multiple concomitant motor-driven signals to ensure the fidelity of the system, while the second model based on frequency-encoding appears to be more robust [16].

Although the model above-mentioned was meant to explain how cells sense their length, similar mechanisms based on oscillatory signals might be used in injury/regeneration. Indeed,

oscillations in the nuclear import of certain transcriptional factors, including Smad1 and STAT3, which play an important role in injury response as previously discussed, has been demonstrated to modulate gene expression in various biological systems [100–103]. Such a system might enable lesion sensing as a sudden change in apparent axon length, possibly through frequency encoding of importin-mediated retrograde signals [16]. Indeed, as previously mentioned, the multiplicity of injury/regenerative signals makes it difficult to devise therapeutic strategies, since mimicking the full spectrum of responses to axonal injury is a strenuous task. A deeper understanding of the mechanisms by which cell bodies receive and integrate retrograde injury signals may highlight common signaling nodes for therapeutic targeting in the future.

Acknowledgements

We thank Dr. Dalia Gordon and Shachar Dagan for constructive comments and for critical reading of the document. Our research on these topics is generously supported by the European Research Council (Neurogrowth), the Israel Science Foundation, the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation, the Minerva Foundation, and the Wings for Life Spinal Cord Research Foundation. M.F. is the incumbent of the Chaya Professorial Chair in Molecular Neuroscience at the Weizmann Institute of Science. The authors have no conflicting financial interests.

References

[1] Goldberg, J.L., Klassen, M.P., Hua, Y. and Barres, B.A. (2002) Amacrine-signaled loss of intrinsic axon growth ability by retinal ganglion cells. Science 296, 1860–1864.
[2] Konishi, Y., Stegmüller, J., Matsuda, T., Bonni, S. and Bonni, A. (2004) Cdh1-APC controls axonal growth and patterning in the mammalian brain. Science 303, 1026–1030.
[3] Zou, Y., Chiu, H., Zinovyeva, A., Ambros, V., Chuang, C.-F. and Chang, C. (2013) Developmental decline in neuronal regeneration by the progressive change of two intrinsic timers. Science 340, 372–376.
[4] Liu, K., Tedeschi, A., Park, K.K. and He, Z. (2011) Neuronal intrinsic mechanisms of axon regeneration. Annu. Rev. Neurosci. 34, 131–152.
[5] Pernet, V. and Schwab, M.E. (2014) Lost in the jungle: new hurdles for optic nerve axon regeneration. Trends Neurosci. 37, 381–387.
[6] Silver, J., Schwab, M.E. and Popovich, P.G. (2015) Central nervous system regenerative failure: role of oligodendrocytes, astrocytes, and microglia. Cold Spring Harb. Perspect. Biol. 7, a020602.
[7] Ruschel, J., Hellal, F., Flynn, K.C., Dupraz, S., Elliott, D.A., Tedeschi, A., Bates, M., Sliwinski, C., Brook, G., Dobrint, K., Peitz, M., Brustle, O., Norenberg, M.D., Blesch, A., Weidner, N., Bunge, M.B., Bixby, J.L. and Bradke, F. (2015) Systemic administration of epothilone B promotes axon regeneration after spinal cord injury. Science 348, 347–352.
[8] Aguayo, A.J., David, S. and Bray, G.M. (1981) Influences of the glial environment on the elongation of axons after injury: transplantation studies in adult rodents. J. Exp. Biol. 95, 231–240.
[9] Lu, P., Woodruff, G., Wang, Y., Graham, L., Hunt, M., Wu, D., Boehle, E., Ahmad, R., Poplawski, G., Brock, J., Goldstein, L.B. and Tuszynski, M. (2014) Long-distance axonal growth from human induced pluripotent stem cells after spinal cord injury. Neuron 83, 789–796.
[10] David, S. and Aguayo, A.J. (1985) Axonal regeneration after crush injury of rat central nervous system fibres innervating peripheral nerve grafts. J. Neurocytol. 14, 1–12.
[11] De Lima, S., Koriyama, Y., Kurimoto, T., Oliveira, J.T., Yin, Y., Li, Y., Gilbert, H.-Y., Fagiolini, M., Martinez a, M.B. and Benowitz, L. (2012) Full-length axon regeneration in the adult mouse optic nerve and partial recovery of simple visual behaviors. Proc. Natl. Acad. Sci. 109, 9149–9154.
[12] Richardson, P.M. and Verge, V.M. (1987) Axonal regeneration in dorsal spinal roots is accelerated by peripheral axonal transection. Brain Res. 411, 406–408.
[13] Neumann, S. and Woolf, C.J. (1999) Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury. Neuron 23, 83–91.
[14] Hoffman, P.N. (2010) A conditioning lesion induces changes in gene expression and axonal transport that enhance regeneration by increasing the intrinsic growth state of axons. Exp. Neurol. 223, 11–18.
[15] Smith, D.S. and Skene, J.H. (1997) A transcription-dependent switch controls competence of adult neurons for distinct modes of axon growth. J. Neurosci. 17, 646–658.
[16] Rishal, I. and Fainzilber, M. (2014) Axon–soma communication in neuronal injury. Nat. Rev. Neurosci. 15, 32–42.
[17] Ghosh-Roy, A., Wu, Z., Goncharov, A., Jin, Y. and Chisholm, A.D. (2010) Calcium and cyclic AMP promote axonal regeneration in *Caenorhabditis elegans* and require DLK-1 kinase. J. Neurosci. 30, 3175–3183.
[18] Mandolesi, G., Madeddu, F., Bozzi, Y., Maffei, L. and Ratto, G.M. (2004) Acute physiological response of mammalian central neurons to axotomy: ionic regulation and electrical activity. FASEB J. 18, 1934–1936.
[19] Sattler, R., Tymianski, M., Feyaz, I., Hafner, M. and Tator, C.H. (1996) Voltage-sensitive calcium channels mediate calcium entry into cultured mammalian sympathetic neurons following neurite transection. Brain Res. 719, 239–246.
[20] Chierzi, S., Ratto, G.M., Verma, P. and Fawcett, J.W. (2005) The ability of axons to regenerate their growth cones depends on axonal type and age, and is regulated by calcium, cAMP and ERK. Eur. J. Neurosci. 21, 2051–2062.
[21] Kamber, D., Erez, H. and Spira, M.E. (2009) Local calcium-dependent mechanisms determine whether a cut axonal end assembles a retarded endbulb or competent growth cone. Exp. Neurol. 219, 112–125.
[22] Neumann, S., Bradke, F., Tessier-Lavigne, M. and Basbaum, A.I. (2002) Regeneration of sensory axons within the injured spinal cord induced by intraganglionic cAMP elevation. Neuron 34, 885–893.
[23] Lu, P., Yang, H., Jones, L.L., Filbin, M.T. and Tuszynski, M.H. (2004) Combinatorial therapy with neurotrophins and cAMP promotes axonal regeneration beyond sites of spinal cord injury. J. Neurosci. 24, 6402–6409.
[24] Park, K., Luo, J.-M., Hisheh, S., Harvey, A.R. and Cui, Q. (2004) Cellular mechanisms associated with spontaneous and ciliary neurotrophic factor-cAMP-induced survival and axonal regeneration of adult retinal ganglion cells. J. Neurosci. 24, 10806–10815.
[25] Kurimoto, T., Yin, Y., Omura, K., Gilbert, H., Kim, D., Cen, L.-P., Moko, L., Kügler, S. and Benowitz, L.I. (2010) Long-distance axon regeneration in the mature optic nerve: contributions of oncomodulin, cAMP, and pten gene deletion. J. Neurosci. 30, 15654–15663.
[26] Cho, Y., Sloutsky, R., Naegle, K.M. and Cavalli, V. (2013) Injury-Induced HDAC5 nuclear export is essential for axon regeneration. Cell 155, 894–908.
[27] Cho, Y. and Cavalli, V. (2012) HDAC5 is a novel injury-regulated tubulin deacetylase controlling axon regeneration. EMBO J. 31, 3063–3078.
[28] Bradke, F., Fawcett, J.W. and Spira, M.E. (2012) Assembly of a new growth cone after axotomy: the precursor to axon regeneration. Nat. Rev. Neurosci. 13, 183–193.
[29] Ertürk, A., Hellal, F., Enes, J. and Bradke, F. (2007) Disorganized microtubules underlie the formation of retraction bulbs and the failure of axonal regeneration. J. Neurosci. 27, 9169–9180.
[30] Erez, H., Malkinson, G., Prager-Khoutorsky, M., De Zeeuw, C.I., Hoogenraad, C.C. and Spira, M.E. (2007) Formation of microtubule-based traps controls the sorting and concentration of vesicles to restricted sites of regenerating neurons after axotomy. J. Cell Biol. 176, 497–507.
[31] Rivieccio, M.A., Brochier, C., Willis, D.E., Walker, B.A., D'Annibale, M.A., McLaughlin, K., Siddiq, A., Kozikowski, A.P., Jaffrey, S.R., Twiss, J.L., Ratan, R.R. and Langley, B. (2009) HDAC6 is a target for protection and regeneration following injury in the nervous system. Proc. Natl. Acad. Sci. U.S.A. 106, 19599–19604.
[32] Gumy, L.F., Chew, D.J., Tortosa, E., Katrukha, E.A., Kapitein, L.C., Tolkovsky, A.M., Hoogenraad, C.C. and Fawcett, J.W. (2013) The kinesin-2 family member KIF3C regulates microtubule dynamics and is required for axon growth and regeneration. J. Neurosci. 33, 11329–11345.
[33] Lv, L., Han, X., Sun, Y., Wang, X. and Dong, Q. (2012) Valproic acid improves locomotion in vivo after SCI and axonal growth of neurons in vitro. Exp. Neurol. 233, 783–790.
[34] Finelli, M., Wong, J. and Zou, H. (2013) Epigenetic regulation of sensory axon regeneration after spinal cord injury. J. Neurosci. 33, 19664–19676.
[35] Saijilafu, Hur E.-M., Liu, C.-M., Jiao, Z., Xu, W.-L. and Zhou, F.-Q. (2013) PI3K-GSK3 signalling regulates mammalian axon regeneration by inducing the expression of Smad1. Nat. Commun. 4, 2690.
[36] Tedeschi, A., Nguyen, T., Puttagunta, R., Gaub, P. and Di Giovanni, S. (2009) A p53-CBP/p300 transcription module is required for GAP-43 expression, axon outgrowth, and regeneration. Cell Death Differ. 16, 543–554.
[37] Gaub, P., Tedeschi, A., Puttagunta, R., Nguyen, T., Schmandke, A. and Di Giovanni, S. (2010) HDAC inhibition promotes neuronal outgrowth and counteracts growth cone collapse through CBP/p300 and P/CAF-dependent p53 acetylation. Cell Death Differ. 17, 1392–1408.
[38] Marvaldi, L., Thongrong, S., Kozłowska, A., Irschick, R., Pritz, C.O., Bäumer, B., Ronchi, G., Geuna, S., Hausott, B. and Klimaschewski, L. (2015) Enhanced axon outgrowth and improved long-distance axon regeneration in sprouty2 deficient mice. Dev. Neurobiol. 75, 217–231.
[39] Gaub, P., Joshi, Y., Wuttke, A., Naumann, U., Schnichels, S., Heiduschka, P. and Di Giovanni, S. (2011) The histone acetyltransferase p300 promotes intrinsic axonal regeneration. Brain 134, 2134–2148.
[40] Puttagunta, R., Tedeschi, A., Sória, M.G., Hervera, A., Lindner, R., Rathore, K.I., Gaub, P., Joshi, Y., Nguyen, T., Schmandke, A., Laskowski, C.J., Boutillier, A.-L., Bradke, F. and Di Giovanni, S. (2014) PCAF-dependent epigenetic changes promote axonal regeneration in the central nervous system. Nat. Commun. 5, 3527.
[41] Ambron, R.T., Dulin, M.F., Zhang, X.P., Schmied, R. and Walters, E.T. (1995) Axoplasm enriched in a protein mobilized by nerve injury induces memory-like alterations in Aplysia neurons. J. Neurosci. 15, 3440–3446.

[42] Schmied, R. and Ambron, R.T. (1997) A nuclear localization signal targets proteins to the retrograde transport system, thereby evading uptake into organelles in *aplysia* axons. J. Neurobiol. 33, 151–160.

[43] Hanz, S., Perlson, E., Willis, D., Zheng, J.Q., Massarwa, R., Huerta, J.J., Koltzenburg, M., Kohler, M., Van-Minnen, J., Twiss, J.L. and Fainzilber, M. (2003) Axoplasmic importins enable retrograde injury signaling in lesioned nerve. Neuron 40, 1095–1104.

[44] Yudin, D., Hanz, S., Yoo, S., Iavnilovitch, E., Willis, D., Gradus, T., Vuppalanchi, D., Segal-ruder, Y., Ben-yaakov, K., Hieda, M., Yoneda, Y., Twiss, J.L. and Fainzilber, M. (2008) Localized regulation of axonal RanGTPase controls retrograde injury signaling in peripheral nerve. Neuron 59, 241–252.

[45] Perlson, E., Hanz, S., Ben-Yaakov, K., Segal-Ruder, Y., Seger, R. and Fainzilber, M. (2005) Vimentin-dependent spatial translocation of an activated MAP kinase in injured nerve. Neuron 45, 715–726.

[46] Cavalli, V., Kujala, P., Klumperman, J. and Goldstein, L.S.B. (2005) Sunday driver links axonal transport to damage signaling. J. Cell Biol. 168, 775–787.

[47] Abe, N., Almenar-Queralt, A., Lillo, C., Shen, Z., Lozach, J., Briggs, S.P., Williams, D.S., Goldstein, L.S.B. and Cavalli, V. (2009) Sunday driver interacts with two distinct classes of axonal organelles. J. Biol. Chem. 284, 34628–34639.

[48] Barnat, M., Enslen, H., Propst, F., Davis, R.J., Soares, S. and Nothias, F. (2010) Distinct roles of c-Jun N-terminal kinase isoforms in neurite initiation and elongation during axonal regeneration. J. Neurosci. 30, 7804–7816.

[49] Tedeschi, A. (2012) Tuning the orchestra: transcriptional pathways controlling axon regeneration. Front. Mol. Neurosci. 4, 60.

[50] Raivich, G., Bohatschek, M., Da Costa, C., Iwata, O., Galiano, M., Hristova, M., Nateri, A.S., Makwana, M., Riera-Sans, L., Wolfer, D.P., Lipp, H.P., Aguzzi, A., Wagner, E.F. and Behrens, A. (2004) The AP-1 transcription factor c-Jun is required for efficient axonal regeneration. Neuron 43, 57–67.

[51] Jankowski, M.P., McIlwrath, S.L., Jing, X., Cornuet, P.K., Salerno, K.M., Koerber, H.R. and Albers, K.M. (2009) Sox11 transcription factor modulates peripheral nerve regeneration in adult mice. Brain Res. 1256, 43–54.

[52] Seijffers, R., Allchorne, A.J. and Woolf, C.J. (2006) The transcription factor ATF-3 promotes neurite outgrowth. Mol. Cell. Neurosci. 32, 143–154.

[53] Seijffers, R., Mills, C.D. and Woolf, C.J. (2007) ATF3 increases the intrinsic growth state of DRG neurons to enhance peripheral nerve regeneration. J. Neurosci. 27, 7911–7920.

[54] Tsujino, H., Kondo, E., Fukuoka, T., Dai, Y., Tokunaga, A., Miki, K., Yonenobu, K., Ochi, T. and Noguchi, K. (2000) Activating transcription factor 3 (ATF3) induction by axotomy in sensory and motoneurons: a novel neuronal marker of nerve injury. Mol. Cell. Neurosci. 15, 170–182.

[55] Schwaiger, F.W., Hager, G., Schmitt, A.B., Horvat, A., Hager, G., Streif, R., Spitzer, C., Gamal, S., Breuer, S., Brook, G.A., Nacimiento, W. and Kreutzberg, G.W. (2000) Peripheral but not central axotomy induces changes in Janus kinases (JAK) and signal transducers and activators of transcription (STAT). Eur. J. Neurosci. 12, 1165–1176.

[56] Ben-Yaakov, K., Dagan, S.Y., Segal-Ruder, Y., Shalem, O., Vuppalanchi, D., Willis, D.E., Yudin, D., Rishal, I., Rother, F., Bader, M., Blesch, A., Pilpel, Y., Twiss, J.L. and Fainzilber, M. (2012) Axonal transcription factors signal retrogradely in lesioned peripheral nerve. EMBO J. 31, 1350–1363.

[57] Qiu, J., Cafferty, W.B.J., McMahon, S.B. and Thompson, S.W.N. (2005) Conditioning injury-induced spinal axon regeneration requires signal transducer and activator of transcription 3 activation. J. Neurosci. 25, 1645–1653.

[58] Shin, J.E., Cho, Y., Beirowski, B., Milbrandt, J., Cavalli, V. and DiAntonio, A. (2012) Dual leucine zipper kinase is required for retrograde injury signaling and axonal regeneration. Neuron 74, 1015–1022.

[59] Valakh, V., Frey, E., Babetto, E., Walker, L.J. and DiAntonio, A. (2015) Cytoskeletal disruption activates the DLK/JNK pathway, which promotes axonal regeneration and mimics a preconditioning injury. Neurobiol. Dis. 77, 13–25.

[60] Lindwall, C. and Kanje, M. (2005) Retrograde axonal transport of JNK signaling molecules influence injury induced nuclear changes in p-c-Jun and ATF3 in adult rat sensory neurons. Mol. Cell. Neurosci. 29, 269–282.

[61] Bareyre, F.M., Garzorz, N., Lang, C., Misgeld, T., Büning, H. and Kerschensteiner, M. (2011) In vivo imaging reveals a phase-specific role of STAT3 during central and peripheral nervous system axon regeneration. Proc. Natl. Acad. Sci. U.S.A. 108, 6282–6287.

[62] Deng, K., He, H., Qiu, J., Lorber, B., Bryson, J.B. and Filbin, M.T. (2009) Increased synthesis of spermidine as a result of upregulation of arginase I promotes axonal regeneration in culture and in vivo. J. Neurosci. 29, 9545–9552.

[63] Ma, T.C., Barco, A., Ratan, R.R. and Willis, D.E. (2014) CAMP-responsive element-binding protein (CREB) and cAMP Co-regulate activator protein 1 (AP1)-dependent regeneration-associated gene expression and neurite growth. J. Biol. Chem. 289, 32914–32925.

[64] Zheng, J.Q., Kelly, T.K., Chang, B., Ryazantsev, S., Rajasekaran, A.K., Martin, K.C. and Twiss, J.L. (2001) A functional role for intra-axonal protein synthesis during axonal regeneration from adult sensory neurons. J. Neurosci. 21, 9291–9303.

[65] Verma, P., Chierzi, S., Codd, A.M., Campbell, D.S., Meyer, R.L., Holt, C.E. and Fawcett, J.W. (2005) Axonal protein synthesis and degradation are necessary for efficient growth cone regeneration. J. Neurosci. 25, 331–342.

[66] Gumy, L.F., Yeo, G.S.H., Tung, Y.-C.L., Zivraj, K.H., Willis, D., Coppola, G., Lam, B.Y.H., Twiss, J.L., Holt, C.E. and Fawcett, J.W. (2011) Transcriptome analysis of embryonic and adult sensory axons reveals changes in mRNA repertoire localization. RNA 17, 85–98.

[67] Welshhans, K. and Bassell, G.J. (2011) Netrin-1-induced local β-actin synthesis and growth cone guidance requires zipcode binding protein 1. J. Neurosci. 31, 9800–9813.

[68] Patel, V.L., Mitra, S., Harris, R., Buxbaum, A.R., Lionnet, T., Brenowitz, M., Girvin, M., Levy, M., Almo, S.C., Singer, R.H. and Chao, J.A. (2012) Spatial arrangement of an RNA zipcode identifies mRNAs under post-transcriptional control. Genes Dev. 26, 43–53.

[69] Willis, D.E., Xu, M., Donnelly, C.J., Tep, C., Kendall, M., Erenstheyn, M., English, A.W., Schanen, N.C., Kirn-Safran, C.B., Yoon, S.O., Bassell, G.J. and Twiss, J.L. (2011) Axonal localization of transgene mRNA in mature PNS and CNS neurons. J. Neurosci. 31, 14481–14487.

[70] Donnelly, C.J., Willis, D.E., Xu, M., Tep, C., Jiang, C., Yoo, S., Schanen, N.C., Kirn-Safran, C.B., van Minnen, J., English, A., Yoon, S.O., Bassell, G.J. and Twiss, J.L. (2011) Limited availability of ZBP1 restricts axonal mRNA localization and nerve regeneration capacity. EMBO J. 30, 4665–4677.

[71] Donnelly, C.J., Park, M., Spillane, M., Yoo, S., Pacheco, A., Gomes, C., Vuppalanchi, D., McDonald, M., Kim, H.H., Merianda, T.T., Gallo, G. and Twiss, J.L. (2013) Axonally synthesized-actin and GAP-43 proteins support distinct modes of axonal growth. J. Neurosci. 33, 3311–3322.

[72] Perry, R.B., Doron-mandel, E., Iavnilovitch, E., Rishal, I., Shachar, Y., Tsoory, M., Coppola, G., Mcdonald, M.K., Gomes, C., Geschwind, D.H., Twiss, J.L., Yaron, A. and Fainzilber, M. (2012) Subcellular knockout of importin β1 perturbs axonal retrograde signaling. Neuron 75, 294–305.

[73] Park, K.K., Liu, K., Hu, Y., Smith, P.D., Wang, C., Cai, B., Xu, B., Connolly, L., Kramvis, I., Sahin, M. and He, Z. (2008) Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Science 322, 963–966.

[74] Liu, K., Lu, Y., Lee, J.K., Samara, R., Willenberg, R., Sears-Kraxberger, I., Tedeschi, A., Park, K.K., Jin, D., Cai, B., Xu, B., Connolly, L., Steward, O., Zheng, B. and He, Z. (2010) PTEN deletion enhances the regenerative ability of adult corticospinal neurons. Nat. Neurosci. 13, 1075–1081.

[75] Morita, M., Gravel, S.-P., Hulea, L., Larsson, O., Pollak, M., St-Pierre, J. and Topisirovic, I. (2015) mTOR coordinates protein synthesis, mitochondrial activity and proliferation. Cell Cycle 14, 473–480.

[76] Xie, J. and Proud, C.G. (2013) Crosstalk between mTOR complexes. Nat. Cell Biol. 15, 1263–1265.

[77] Proud, C.G. (2013) MTORC1 regulates the efficiency and cellular capacity for protein synthesis. Biochem. Soc. Trans. 41, 923–926.

[78] Van Diepen, M.T., Parsons, M., Downes, C.P., Leslie, N.R., Hindges, R. and Eickholt, B.J. (2009) MyosinV controls PTEN function and neuronal cell size. Nat. Cell Biol. 11, 1191–1196.

[79] Abe, N., Borson, S.H., Gambello, M.J., Wang, F. and Cavalli, V. (2010) Mammalian Target of Rapamycin (mTOR) activation increases axonal growth capacity of injured peripheral nerves. J. Biol. Chem. 285, 28034–28043.

[80] Yang, L., Miao, L., Liang, F., Huang, H., Teng, X., Li, S., Nuriddinov, J., Selzer, M.E. and Hu, Y. (2014) The mTORC1 effectors S6K1 and 4E-BP play different roles in CNS axon regeneration. Nat. Commun. 5, 5416.

[81] Duan, X., Qiao, M., Bei, F., Kim, I.-J., He, Z. and Sanes, J.R. (2015) Subtype-specific regeneration of retinal ganglion cells following axotomy: effects of osteopontin and mTOR signaling. Neuron 85, 1244–1256.

[82] Ahmed, M. and Kundu, G.C. (2010) Osteopontin selectively regulates p70S6K/mTOR phosphorylation leading to NF-kappaB dependent AP-1-mediated ICAM-1 expression in breast cancer cells. Mol. Cancer 9, 101.

[83] Christie, K.J., Webber, C.A., Martinez, J.A., Singh, B. and Zochodne, D.W. (2010) PTEN inhibition to facilitate intrinsic regenerative outgrowth of adult peripheral axons. J. Neurosci. 30, 9306–9315.

[84] Cho, Y., Di Liberto, V., Carlin, D., Abe, N., Li, K.H., Burlingame, A.L., Guan, S., Michaelevski, I. and Cavalli, V. (2014) Syntaxin13 expression is regulated by mammalian target of rapamycin (mTOR) in injured neurons to promote axon regeneration. J. Biol. Chem. 289, 15820–15832.

[85] Fischer, D., Pavlidis, M. and Thanos, S. (2000) Cataractogenic lens injury prevents traumatic ganglion cell death and promotes axonal regeneration both in vivo and in culture. Invest. Ophthalmol. Vis. Sci. 41, 3943–3954.

[86] Fischer, D., Petkova, V., Thanos, S. and Benowitz, L.I. (2004) Switching mature retinal ganglion cells to a robust growth state in vivo: gene expression and synergy with RhoA inactivation. J. Neurosci. 24, 8726–8740.

[87] Smith, P.D., Sun, F., Park, K.K., Cai, B., Wang, C., Kuwako, K., Martinez-Carrasco, I., Connolly, L. and He, Z. (2009) SOCS3 deletion promotes optic nerve regeneration in vivo. Neuron 64, 617–623.

[88] Qin, S., Zou, Y. and Zhang, C.-L. (2013) Cross-talk between KLF4 and STAT3 regulates axon regeneration. Nat. Commun. 4, 2633.

[89] Sun, F., Park, K.K., Belin, S., Wang, D., Lu, T., Chen, G., Zhang, K., Yeung, C., Feng, G., Yankner, B.A. and He, Z. (2011) Sustained axon regeneration induced by co-deletion of PTEN and SOCS3. Nature 480, 372–375.

[90] Michaelevski, I., Segal-Ruder, Y., Rozenbaum, M., Medzihradszky, K.F., Shalem, O., Coppola, G., Horn-Saban, S., Ben-Yaakov, K., Dagan, S.Y., Rishal, I., Geschwind, D.H., Pilpel, Y., Burlingame, A.L. and Fainzilber, M. (2010) Signaling to transcription networks in the neuronal retrograde injury response. Sci. Signal. 3, ra53.

[91] Chen, L., Wang, Z., Ghosh-Roy, A., Hubert, T., Yan, D., O'Rourke, S., Bowerman, B., Wu, Z., Jin, Y. and Chisholm, A.D. (2011) Axon regeneration pathways identified by systematic genetic screening in *C. elegans*. Neuron 71, 1043–1057.

[92] Kenney, A.M. and Kocsis, J.D. (1998) Peripheral axotomy induces long-term c-Jun amino-terminal kinase-1 activation and activator protein-1 binding

activity by c-Jun and junD in adult rat dorsal root ganglia in vivo. J. Neurosci. 18, 1318–1328.
[93] You, S.W., So, K.F. and Yip, H.K. (2000) Axonal regeneration of retinal ganglion cells depending on the distance of axotomy in adult hamsters. Invest. Ophthalmol. Vis. Sci. 41, 3165–3170.
[94] Kholodenko, B.N. (2003) Four-dimensional organization of protein kinase signaling cascades: the roles of diffusion, endocytosis and molecular motors. J. Exp. Biol. 206, 2073–2082.
[95] Markevich, N.I., Tsyganov, M.A., Hoek, J.B. and Kholodenko, B.N. (2006) Long-range signaling by phosphoprotein waves arising from bistability in protein kinase cascades. Mol. Syst. Biol. 2, 61.
[96] Stelling, J. and Kholodenko, B.N. (2009) Signaling cascades as cellular devices for spatial computations. J. Math. Biol. 58, 35–55.
[97] Kam, N., Pilpel, Y. and Fainzilber, M. (2009) Can molecular motors drive distance measurements in injured neurons? PLoS Comput. Biol. 5, e1000477.
[98] Rishal, I., Kam, N., Perry, R.B.T., Shinder, V., Fisher, E.M.C., Schiavo, G. and Fainzilber, M. (2012) A motor-driven mechanism for cell-length sensing. Cell Rep. 1, 608–616.
[99] Kuznetsov, A.V. (2013) An analytical solution describing the propagation of positive injury signals in an axon: effect of dynein velocity distribution. Comput. Methods Biomech. Biomed. Eng. 16, 699–706.
[100] Cai, L., Dalal, C.K. and Elowitz, M.B. (2008) Frequency-modulated nuclear localization bursts coordinate gene regulation. Nature 455, 485–490.
[101] Wee, K.B., Yio, W.K., Surana, U. and Chiam, K.H. (2012) Transcription factor oscillations induce differential gene expressions. Biophys. J. 102, 2413–2423.
[102] Yoshiura, S., Ohtsuka, T., Takenaka, Y., Nagahara, H., Yoshikawa, K. and Kageyama, R. (2007) Ultradian oscillations of Stat, Smad, and Hes1 expression in response to serum. Proc. Natl. Acad. Sci. U.S.A. 104, 11292–11297.
[103] George, E.B., Glass, J.D. and Griffin, J.W. (1995) Axotomy-induced axonal degeneration is mediated by calcium influx through ion-specific channels. J. Neurosci. 15, 6445–6452.