Cell intrinsic control of axon regeneration

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Abstract

Although neurons execute a cell intrinsic program of axonal growth during development, following the establishment of connections, the developmental growth capacity declines. Besides environmental challenges, this switch largely accounts for the failure of adult central nervous system (CNS) axons to regenerate. Here, we discuss the cell intrinsic control of axon regeneration, including not only the regulation of transcriptional and epigenetic mechanisms, but also the modulation of local protein translation, retrograde and anterograde axonal transport, and microtubule dynamics. We further explore the causes underlying the failure of CNS neurons to mount a vigorous regenerative response, and the paradigms demonstrating the activation of cell intrinsic axon growth programs. Finally, we present potential mechanisms to support axon regeneration, as these may represent future therapeutic approaches to promote recovery following CNS injury and disease.

Keywords axon regeneration; axonal transport; conditioning lesion; microtubule dynamics

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See the Glossary for abbreviations used in this article.

Introduction

During development, by implementing a transcription-dependent program relying on multiple signaling pathways, neurons display robust elongation capacity that allows them to reach their targets. Following this initial phase, with the establishment of connections the developmental axon elongation ability declines. The view that neurons in the adult CNS permanently lose their intrinsic ability to grow axons was challenged by seminal studies by the Albert Aguayo group, showing that the use of peripheral nerve “bridges” in the spinal cord permits CNS axons to grow for considerable distances following injury [1]. These studies demonstrated that adult CNS neurons can activate a cell intrinsic program that supports axonal regrowth, provided that a permissive environment is created. These initial reports fueled efforts to characterize the extrinsic cues that inhibit axon growth in the CNS, while the cell intrinsic mechanisms that govern axon regeneration remained poorly understood. Several decades later, the body of knowledge gathered supports the view that countering or removing the extracellular inhibitory molecules results in incomplete axon regeneration in vivo and that a better understanding of cell intrinsic mechanisms regulating axon growth following injury is needed [2].

In contrast to the CNS, adult peripheral nervous system (PNS) axons can spontaneously regrow to a significant extent and are often used as a model to identify the players that promote axon regeneration. The regenerative capacity of the PNS is supported by the combination of extrinsic and intrinsic factors that generate a growth-permissive milieu where the execution of a cell intrinsic program leads to successful axonal regrowth. Cell intrinsic changes induced by a PNS injury can be observed in vitro and in vivo, as will be discussed in the context of the conditioning lesion paradigm. In CNS neurons, the combined action of repressors of axonal growth, the limited injury signaling mechanisms, and the lack of robust expression of regeneration-associated genes (RAGs) results in a restricted potential to regenerate. Here, we will provide a critical perspective of our understanding of the intrinsic mechanisms controlling axonal regeneration in the adult nervous system. With the term cell intrinsic, we refer to mechanisms that do not strictly depend on external cues, although external cues can influence their activity. As such, this review is not restricted to the discussion of changes in the expression of the neuronal genetic program, that is, transcriptional and epigenetic mechanisms and regulation of translation, but is extended to the analyses of intracellular pathways and mechanisms—including axonal transport and microtubule dynamics—that regulate axon growth and regeneration.

Cell intrinsic mechanisms of axonal regeneration in the PNS

Calcium influx into the axoplasm is one of the first signals caused by injury, and the depolarization triggered by the inversion of the calcium/sodium flux travels along the axon to the cell body. Calcium influx is here discussed in the context of the cell intrinsic factors that govern axon regeneration as it elicits various cell autonomous mechanisms necessary for successful axon growth, ranging from the regulation of intracellular pathways to the generation of...
epigenetic changes. In Caenorhabditis elegans sensory neurons, the amplitude of the axonal calcium waves correlates with the extent of regeneration, and conversely, inhibition of voltage-gated calcium channels, or of calcium release from internal stores, reduces the regenerative growth of axons [3]. Although the consequences of electrical stimulation produce conflicting results, possibly due to differences in stimulation paradigms, a weak stimulus may improve the regeneration of rat motor [4] and sensory neurons [5]. However, a strong electrical pulse mimicking the physiological activity of adult rodent dorsal root ganglia (DRG) neurons strongly inhibits axon outgrowth, and loss of electrical activity following PNS injury promotes axonal regeneration in the PNS [6].

Independently of the electrical activity generated by calcium influx, the calcium transient activates intracellular signaling required for rescaling the axonal membrane in giant squid axons [7], for local protein synthesis after optic nerve crush in rats [8,9] and for the assembly of a competent growth cone after axotomy of Aplysia buccal neurons [8,9]. Besides, calcium influx activates calcium-dependent enzymes including adenylate cyclase, leading to increased cAMP levels that signal to the downstream dual leucine zipper kinase (DLK-1) promoting the local transformation of the cytoskeleton needed for growth cone assembly in C. elegans sensory neurons [3] (Fig 1). In mouse sensory neurons, the calcium wave requires calcium release from internal stores in addition to voltage-gated calcium channels [10]. Importantly, this back-propagating calcium wave invades the soma causing protein kinase Cζ (PKCζ) activation followed by nuclear export of histone deacetylase 5 (HDAC5), thereby increasing histone acetylation and activating the proregenerative transcription program [10] (Fig 1). This epigenetic mechanism controls the switch from non-elongating to growth-competent axons [10]. This early and fast calcium-dependent phase of injury signaling has been suggested to prime the neuronal cell body to the signals that will be conveyed later, after microtubule-dependent retrograde transport along the axon [10].

The importance of increasing histone acetylation to induce axonal regeneration has also been demonstrated using the HDAC inhibitor valproic acid, which improves the outcome in a rat model of spinal cord injury [11]. Further reinforcing the link between increased axon growth and histone acetylation, in mouse DRG neurons triggered into a growth state, as is the case following conditioning lesion (this model is discussed below), histone 4 acetylation is restored and RAG transcription is initiated [12]. During this epigenetic reprogramming, histone-modifying enzymes together with Smad1 facilitate the transcriptional activation of RAGs, including neuropeptide Y (NPY), vasointestinal peptide (VIP), Spr1α, and galanin, thus providing a link between transcription factors and RAGs through epigenetic regulation [12]. Importantly, the Smad1 pathway has been recently shown to be central for promoting rat sensory axon regeneration [13]. Several other epigenetic mechanisms have been reported in the context of axon regrowth [14]. The histone acetyltransferases CBP/p300 acetylate histone 3 at K9-14 and the transcription factor p53, thereby initiating a proregenerative transcriptional program that regulates RAG expression in rodents [15–17]. In this context, p300 directly occupies and acetylates histones in the promoters of the growth-associated protein 43 (GAP-43), coronin 1 b, and Spr1α driving the expression of several RAGs [17]. The importance of this mechanism is highlighted by the observation that, in the model of optic nerve crush, overexpression of p300 promotes axonal growth [17]. Besides, axonal regeneration in the rodent CNS after spinal cord injury is dependent on the folate pathway through DNA methylation [18]. A thorough comparison of the epigenetic landscape in regenerative and non-regenerative conditions is required to translate the knowledge gathered in this field into novel therapeutic approaches [19].

Retrograde transport of locally activated injury signals

In addition to calcium-mediated signaling, studies using Aplysia neurons described the first injury signals capable of communicating lesion from the injury site to the cell body [20,21]. In Aplysia, injection of axoplasm from injured nerves into naïve neurons triggers an injury-like behavior accompanied by increased growth. The model proposed to explain this behavior has been that phosphorylation of injury signals exposes hidden nuclear localization signals (NLS),
targeting them to the nucleus [22]. The importance of retrograde transport of NLS-containing proteins has been demonstrated later in rats, as injection of an NLS synthetic peptide into the injured nerve competes with the activation of intrinsic growth programs by preventing the retrograde transport of injury signals [23]. NLS-containing proteins bind with low affinity to importin-α, the only importin present in intact nerves, but with high affinity to importin-α/β heterodimers. Following injury, local translation of importin-β at the injury site leads to the formation of importin-α/β heterodimers, which bind to NLS-containing proteins and are retrogradely transported to the cell body [23]. In fact, axonal localization of importin-β mRNA is essential for the correct assembly of the retrograde transport machinery of injury signals as demonstrated in rodent DRG neurons [24]. In the case of ERK, its binding to the retrograde transport machinery is not dependent on an NLS signal. Instead, ERK is linked to the retrograde transport machinery through locally synthesized vimentin [25] (Fig 2), as further discussed below. Besides importin-β, Ras-related nuclear protein binding protein 1 (RanBP1) is also synthesized locally after injury, allowing the binding of importin-α/β heterodimers to dynein in rat DRG neurons [26]. Below, the importance of local protein translation in axon regeneration will be further discussed.

Several injury signals locally activated and retrogradely transported to the cell body have been identified using mostly rat or mouse DRG neurons and sciatic nerve injury as a model: extracellular signal-regulated kinase (ERK) [25], c-Jun N-terminal kinases (JNK) [27], and signal transducer and activator of transcription 3 (STAT3) [28] (Fig 1). In the case of ERK, the use of MEK1,2 inhibitors following peripheral nerve injury reduces the regenerative response, suggesting that MEK may phosphorylate ERK at the injury site [25]. To overcome the challenge of transporting phosphorylated signals from the injury site to the cell body, protection mechanisms...
are in place. As discussed above, vimentin, for example, binds to phosphorylated ERK, which enables not only linkage to the retrograde transport machinery but also hinders ERK dephosphorylation [25] (Fig 2). Although pERK is involved in the retrograde signal that initiates regeneration, it is probably not required during subsequent outgrowth [25]. Also, the formation of the JNK-Sunday Driver complex allows the signal to be transported on vesicular structures linked to the transport machinery, possibly protecting it from dephosphorylation [29]. Importantly, JNK signaling has been implicated in the reorganization of the axonal cytoskeleton and in neurite regeneration [30]. In the case of STAT3, it is interesting to note that besides contributing to axonal regeneration [31], its activation is also important for neuronal survival after injury [28].

The response to nerve injury relies on the activation of numerous transcription factors. Some of the transcription factors are activated by the above injury signals. ERK activates ETS domain-containing protein (Elk-1) [26], while JNK activates c-Jun and activating transcription factor 3 (ATF3) [32]. Other transcription factors involved in the regenerative response have also been identified in assays using rat and mouse DRG neurons, including cAMP response element-binding protein (CREB) [31], SRY-related HMG-box (Sox11) [33], phosphatidylinositol 3-kinase (PI3K), and Smad1 [13,34]. Together, they alter the transcriptional profile of injured neurons contributing to their survival and regeneration [33]. In this context, and integrating previous data, it has been demonstrated that PNS injury activates PI3K signaling, leading to the inactivation of glycogen synthase kinase 3 (GSK3) and suggesting that the PI3K-GSK3-Smad1 pathway is central for promoting sensory axon regeneration [13]. The activated transcription factors also induce the expression of several RAGs including arginase-1 [34], NPY, VIP [35], interleukin-6 (IL-6) [36], GAP-43, and CAP-23 [37], among others (Fig 1).

As a result of injury, the interruption of retrograde transport of negative injury signals, possibly target-derived molecules, might release neurons from the repression to elongate, allowing regeneration to take place. In this context, it has been demonstrated that following lesion, reduction in nerve growth factor (NGF) levels in sympathetic and sensory neurons contributes to the increased levels of neuropeptide expression [38]. Likewise, cessation of electrical activity after peripheral lesion contributes to the regenerative response [6]. As such, both target-derived NGF and electrical activity are seen as negative injury signals. In summary, in addition to the presence of regeneration-promoting injury signals, in adult naïve PNS neurons repression of axonal elongation might be relieved upon injury.

Anterograde axonal transport for an effective regenerative response

As neurons are highly polarized cells, proteins synthesized as a response to injury signaling need to travel from the cell body to the distant axon tip. Thus, the control of anterograde axonal transport is an intracellular mechanism of pivotal importance for axon regeneration. Anterograde axonal transport is divided into the slow component a (SCa) that transports neurofilaments, tubulin, and microtubule-associated proteins; the SCb that transports cytoplasmic proteins, such as glycolytic enzymes and actin; and the fast component that transports vesicles and membranous organelles [39].

Surprisingly, the motors of both slow and fast components are similar, and the different average rates are due to the pausing behavior of cargoes during transport [40]. The flux of anterograde axonal transport elicited by injury needs to supply the axon with structural components (tubulin, actin, and neurofilaments), synaptic and cytoplasmic proteins, vesicles, and organelles. Interestingly, the speed of axonal regeneration is similar to the one of SCb, supporting the relevance of anterograde transport in sustaining regrowing axons [41]. Of note, following sciotic nerve injury in mice, anterograde transport of mitochondria in the proximal nerve increases by more than 80% and declines only slightly subsequently [42], which may support the increased metabolic demand of regenerating peripheral axons (Fig 2). Whether the transport of other organelles or cytoplasmic proteins is also increased remains to be clarified.

Despite the discussed evidence suggesting that axonal transport plays a central role during axonal regeneration, the modulation of transport by injury is not well understood. Specifically, the mechanisms that underlie the increase in axonal transport after PNS injury remain to be established, and future studies should determine whether molecular motors are affected by lesion or, if alternatively, microtubule tracks are modified.

Zipcodes and local protein synthesis during axonal regeneration

The relevance of local protein synthesis in axons remained obscure until recently. To date, as a consequence of several studies performed mainly in rodents, it is widely accepted that the first building blocks of regenerating axons are obtained by local protein synthesis along the axon and in the growth cone. In the adult PNS, axons contain ribosomes distributed unevenly along the axoplasm [43], and Schwann cells may also provide axonal ribosomes following injury [44]. In contrast, in the CNS, axons synthesize proteins during development in the growth cone, but polysomes are restricted to the axon initial segment in adult rodent axons [45]. Besides the correlation between the different capacities of PNS and CNS axons to locally synthesize proteins and regenerate, local protein synthesis generally decreases with axonal aging, which again coincides with a reduced regeneration potential [46]. Further supporting a critical role for local protein synthesis during axonal regeneration, application of inhibitors of protein synthesis to cut rat axons, including axons whose cell bodies were removed, decreases the number of transected axons that reform a growth cone [45]. In fact, growth cone formation after axotomy depends on local protein synthesis and degradation, controlled by the mammalian target of rapamycin (mTOR), p38MAPK, and caspase-3-dependent pathways [45].

The identification of axonally localized mRNAs has been facilitated considerably with the development of more sensitive techniques. Genome-wide microarray analyses revealed that several mRNAs are localized axonally in rat sensory axons and that this repertoire changes substantially from development to adulthood [47,48]. Axonal mRNAs need to be actively transported, stored, and protected from degradation at their final destination. The β-actin zipcode, a conserved sequence present at the beginning of its 3′ UTR, is the sequence for mRNA axonal targeting identified in mice. [49]. This sequence interacts with the RNA-binding protein zipcode-binding protein 1 (ZBP-1), which mediates the axonal
localization of β-actin [50] (Fig 2). Reinforcing the importance of this mechanism for axonal regrowth, mice with reduced ZBP1 levels show decreased axon regeneration after sciatic nerve injury [51]. Additionally, the overexpression of β-actin’s 3′ UTR was shown to compete in vivo with other ZBP1 cargo mRNAs such as GAP-43 [52]. It has now been demonstrated that axonal translation of β-actin supports axon branching, while that of GAP-43 promotes the elongation of rodent sensory neurons during normal axon growth [53]. These growth-promoting pathways might be relevant for regenerating axons as well. As already addressed, data supporting the local axonal translation of importin β1 have been obtained and an axon-localizing region in the 3′ UTR of importin β1 has been identified [24]. Mice lacking the axon-localizing region in the 3′ UTR of importin β1 displayed a delay in axonal regeneration of sensory neurons [24]. In summary, the above findings support the conclusion that the ability of axons to locally synthesize proteins is important for their capacity to regenerate.

Why are CNS neurons unable to mount a robust regenerative program?

In contrast to the PNS, injured CNS axons have a limited ability to regenerate. Besides the formation of a highly inhibitory glial scar, several differences can be put forward to explain this lack of regenerative capacity, including inefficient Wallerian degeneration, possible defects in injury signaling, lack of a robust response to injury, limited capacity to locally synthesize proteins, and the existence of inhibitors of axonal regrowth. Indeed, rat CNS neurons fail to effectively activate many of the genes necessary for axonal regeneration to occur [54]. Interestingly, the calcium changes in the cell body have a higher amplitude and duration in rat DRG when compared to cortical neurons [55], and DRG neurons can survive long periods of high calcium, whereas these are deleterious for CNS neurons [56]. Besides, increased histone acetylation fails to occur in retinal ganglion cells (RGCs) [10]. Together, these differences might contribute to the failure in activating a proregenerative program (Fig 3).

Significant advances have been made with the identification of intrinsic inhibitors of axon regrowth in the adult CNS in studies mainly performed by gene targeting in mice. In adult RGCs, deletion of phosphatase and tensin homolog (PTEN) promotes robust axon regeneration after optic nerve injury [57]. In the PNS, following nerve transection, adult sensory neurons depleted of PTEN also show increased axon regeneration [58]. PTEN antagonizes the action of PI3K, leading to the inactivation of protein kinase B (AKT) and of mTOR signaling. In contrast to the CNS, mTOR has been suggested to be dispensable for sensory axon regeneration [58], where instead, the PI3K-GSK3-Smad1 pathway operates [13]. However, the importance of mTOR in PNS regeneration remains to be clarified as in contrast to CNS neurons, which downregulate mTOR activity after injury, PNS neurons activate mTOR and deletion of tuberous sclerosis complex 2 (TSC2), another negative regulator of mTOR, increases sensory axon regeneration in vivo [59]. It is noteworthy that the observation that mTOR might be dispensable for sensory axon regeneration under physiological conditions does not necessarily contradict the result that ectopic activation of mTOR (as occurs after the deletion of TSC2) promotes axon regeneration.

Through the analysis of axon regeneration in different mutant mouse lines, deletion of the suppressor of cytokine signaling 3 (SOCS3), an inhibitor of the JAK-STAT3 pathway, has been shown to promote robust regeneration of injured optic nerve axons [60]. Of note, simultaneous deletion of PTEN and SOCS3 further increases

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**Figure 3. Increased growth capacity of PNS versus CNS neurons.**

During development, through a cell intrinsic program composed of multiple pathways, neurons display a robust elongation capacity. After the establishment of connections, adult CNS neurons have a limited regenerative ability as the consequence of decreased calcium changes, no increase in histone acetylation, lack of robust synthesis of RAGs, limited local protein synthesis, and presence of inhibitors of axon regrowth (PTEN, SOCS3, and EFA-6). Adult PNS neurons have, however, a high intrinsic growth capacity as a consequence of the activation of a regeneration-specific program and probably also through the recapitulation of developmental-related pathways. CNS, central nervous system; EFA-6, exchange factor for Arf6; PNS, peripheral nervous system; PTEN, phosphatase and tensin homolog; RAG, regeneration-associated gene; SOCS3, suppressor of cytokine signaling 3.
axon regeneration, as these signals regulate two independent pathways that act synergistically [61]. In summary, the mTOR and STAT3 pathways emerged as key regulators promoting long-distance axon regeneration in the adult CNS. Reinforcing this view, PTEN or SOCS3 deletion improves regeneration of distinct CNS axons [57,60,62,63], and sustained activation of STAT3 promotes corticospinal remodeling and functional recovery after spinal cord injury [64]. Recently, in C. elegans mechanosensory neurons, the conserved Arf guanine nucleotide-exchange factor EFA-6 has been reported to be an intrinsic inhibitor of axon regeneration that operates by affecting axonal microtubule dynamics, acting downstream of and/or in parallel with DLK-1 [65].

Together, these studies indicate that axon regeneration is restrained not only by extrinsic inhibitory cues but also by intrinsic factors. As such, the manipulation of intrinsic growth control pathways is actively being pursued as a therapeutic approach to promote axon regeneration after CNS injury.

The cell intrinsic growth capacity of adult CNS neurons can be activated

Despite the general inability of CNS axons to regenerate, regrowth can be activated under specific conditions, for example the conditioning lesion effect. DRG neurons possess a peripheral axon branch that regenerates when injured, and a central axon branch that enters the spinal cord (forming the dorsal column fibers) and does not regenerate upon injury. However, when the peripheral branch is lesioned prior to the conditioning lesion, the central axon can overcome the glial scar inhibitory effect, regenerating to a significant extent both in vivo and in vitro [66,67], when plated on inhibitory substrates, including myelin [68]. The conditioning effect is probably due to the activation of the regenerative machinery prior to CNS lesion. Studies performed in rodents have shown that the increase in regeneration capacity encompasses RAG expression [33,69] and possibly changes in axonal transport [70]. In fact, the response to injury starts as soon as 1 day following lesion [68] and has a long-lasting effect, as RAGs are still expressed 2 months following the priming injury [71]. Although a peripheral lesion performed subsequently to the CNS injury does not improve axonal regeneration due to the assembly of a thick glial scar, it still increases the intrinsic regenerative ability of DRG neurons [71].

The conditioning effect certainly represents a good model to identify the mechanisms underlying the cell intrinsic regenerative capacity. Numerous molecular pathways have been shown to be regulated by a conditioning lesion, in accordance with the robust and broad transcriptional change that conditioning causes in DRG neurons. The initial unifying concept was that the conditioning effect was mediated by increased cAMP levels induced by injury [68]. cAMP prompted neurons to overcome myelin inhibition in vitro, and treatment with dibutyryl-cAMP, a cell-permeable analog of cAMP, increased the regeneration of dorsal column fibers following spinal cord injury [68,72]. Further supporting the pivotal role of cAMP, the phosphodiesterase inhibitor rolipram increases cAMP levels, leading to enhanced regeneration of serotonergic axons and functional recovery following spinal cord injury in rats [73]. However, the robust effect of cAMP in axon regeneration has been questioned by recent studies, as the use of cAMP analogs has failed to reproduce the full effect of a conditioning lesion [74]. Increased levels of cAMP appear to promote axon regeneration by overcoming myelin-based inhibitors rather than by modulating the intrinsic ability of neurons to support axon regeneration. It has been shown that elevation of cAMP fails to increase the SCb of axonal transport, the rate-limiting step of axon growth [75].

Besides cAMP, several studies have identified broad changes in gene expression in rodent conditioned neurons [36,69], regulated by the activation of multiple transcription factors [76]. Downstream, the expression of traditional RAGs such as GAP-43 and CAP-23 [77] is induced and novel RAGs have been identified using this model, including arginase-1 [34] and IL-6 [36]. However, none of the identified transcription factors or RAGs reproduces the entire conditioning effect [74], suggesting that conditioning cannot be mimicked by manipulating a single pathway. Supporting this view, epigenetic changes elicited by HDAC5 nuclear export partially reproduce the conditioning lesion effect [10]. Several questions remain open, including the differences in injury-induced signaling that allow a peripheral injury to elicit a strong regeneration response, whereas a central lesion to the same neuron fails to do so (Sidebar A).

Manipulating axonal microtubule dynamics to promote regeneration

Among the multiple processes involved in the generation of a new growth cone, cytoskeleton reorganization is crucial for the intrinsic ability to regenerate. While upon injury CNS axons form a retraction bulb with a disorganized network of microtubules, PNS axons form a growth cone with stable microtubules in the backbone and dynamic microtubules in the tip [78]. Pharmacological destabilization of microtubules converts a growth cone into a retraction bulb, and taxol-induced stabilization generates growth cones that can overcome myelin inhibition [78] and regenerate following spinal cord injury [79]. Also, HDAC6 inhibition in rodents results in increased levels of acetylated/stable microtubules and enhances the growth of sensory neurons on myelin [80]. It has been recently suggested that instead of inducing the normal mode of repair, where the axon tip might behave more dynamically, taxol might promote axonal regeneration by enabling the axon tip to become more forceful [81]. In rodent sensory neurons, HDAC5 accumulates at the tip of injured axons where local tubulin deacetylation induces growth cone microtubule dynamics and axon regeneration [82]. The varying effects that microtubule stability might have on axon regeneration do not allow for a clear causal relationship between axon regeneration and microtubule stability. Moreover, at this point, the available literature does not provide sufficient detail to allow for a comparison between the effects of HDAC5, HDAC6, and taxol on tubulin dynamics in the axon shaft versus axon tip. Although the results obtained with studies using taxol and HDAC6 seem contradictory to the data reported for HDAC5 and the kinesin family member KIF3C [83], previous studies have shown that efficient developmental axon growth requires an optimal level of microtubule dynamics. Thus, destabilizing or overstabilizing microtubules could both impair axon growth.
Many of the pathways that contribute to cell intrinsic control of regeneration participate in the remodeling of the axonal cytoskeleton, specifically by modulating microtubule dynamics. As such, potential therapeutic strategies intervening at the level of microtubule-related proteins have been actively pursued. In this respect, and besides those already discussed in this review including HDAC5 and 6, several other possible targets have been identified, including GSK3β, as many of its substrates are microtubule-interacting proteins [84].

Members of the kinesin family have also been put forward as important players in regulating microtubule dynamics during axonal regeneration. KIF3C has been shown to be an injury-specific kinesin with microtubule-stabilizing function, playing a key role during axon regrowth [83]. Depletion of KIF3C in adult neurons leads to an increase in stable and looped microtubules and delays axonal regeneration after injury [83]. In addition to KIF3C, in C. elegans mechanosensory axons, the depolymerizing kinesin-like protein family member 7 (KLP-7) restricts microtubule growth in the steady state [85]. After axon injury, the number of growing microtubules is increased at the injury site, simultaneously with the downregulation of KLP-7, in a cascade coordinated by DLK-1 [85]. This mechanism has been proposed to allow the stable microtubule cytoskeleton of a mature neuron to be converted into the dynamically growing microtubule cytoskeleton of a regenerating axon.

Besides the importance of identifying microtubule-interacting proteins that participate in either the formation of a retraction bulb or of a growth cone, further analysis of the regulation of post-translational microtubule modifications following injury is needed, as these control microtubule dynamics and may also interfere with axonal transport, therefore impacting on axonal regrowth.

Conclusions and outlook

Recent evidence obtained by systematic genetic screening in C. elegans shows that besides triggering developmental programs that may be repressed in mature neurons, regenerative growth involves specific pathways that sense and specifically respond to damage [65] (Fig 3). Although axonal regeneration cannot be viewed as mere recapitulation of axonal elongation during development, some of the operating mechanisms are shared. Axon growth during development occurs in two different phases: an initial phase of growth and a later phase that takes place during pruning [86]. Axon growth during developmental remodeling appears to be mechanistically distinct from initial axon outgrowth. Interestingly, common pathways, for example mTOR signaling, operate during developmental axon regrowth and axonal regeneration [86].

The ubiquitin ligase Cdh1-anaphase-promoting complex (Cdh1-APC) pathway is also a critical cell intrinsic mechanism that regulates axon growth in the rodent developing cerebellar cortex [87]. The inhibition of Cdh1-APC in primary neurons enhances axonal growth and also overrides myelin inhibition of axon regrowth [87]. Conceivably, Cdh1-APC might therefore potentially limit axonal growth in the adult CNS. In key follow-up studies, the transcriptional regulator SnoN has been identified as a critical substrate of Cdh1-APC in neurons [88], operating in a pathway that is regulated by transforming growth factor β-Smad2 signaling [89]. Smad2 knockdown also overrides myelin inhibition of axon growth [89]. More recently, expression of a mutant SnoN resistant to degradation has been shown to enhance axonal regeneration following spinal cord injury in rats [90]. Together, these findings raise the exciting prospect that pathways operating during development might also drive axonal regeneration following injury. Therapeutic strategies aiming at the reactivation of these pathways in injured CNS neurons might be successful in enhancing our capacity to regenerate neurons in response to injury or disease.

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Conflict of interest

The authors declare that they have no conflict of interest.

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activity suppresses axon growth through Ca(v)1.2 channels in adult primary sensory neurons. Curr Biol 20: 1154 – 1164


