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Growing the growth cone: remodeling the cytoskeleton to promote axon regeneration

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Abstract

Axon growth is driven by the movement of a growth cone, a specialized sensory-motile structure located at the tip of a growing neurite. Although stalled retraction bulbs have long been recognized as hallmarks of regeneration failure, mechanisms that control the formation and migration of the nerve endings are only beginning to be unraveled. Recent studies point to microtubules as key determinants for such processes, and emerging evidence suggests that regulators of the actin and microtubule dynamics in the growth cone might serve as attractive targets for controlling both the speed and trajectory of regenerating axons. This review discusses the potential and recent progress of directly modulating the growth cone machinery as a novel strategy to promote axon regeneration in the nervous system after injury.

Introduction

Injury to the central nervous system (CNS) often results in permanent deficits because axons fail to undergo regeneration, which is attributed to both extrinsic and intrinsic factors. Over the last three decades, extensive focus has been on the characterization of extrinsic factors comprising the CNS environment, leading to the identification of a growing list of axon growth impediments, such as myelin-based inhibitors and components in the glial scar [1–3]. Downstream signaling events that convey axon growth inhibition have also been investigated [1–5], with the hope of formulating axon regeneration strategies by alleviating the inhibitory influences (Figure 1A). However, although some issues still remain controversial [6–8], recent studies suggest that counteracting the inhospitable milieu alone is insufficient to trigger long-distance axon regeneration [5, 9–12].

Another obstacle to axon regeneration is the age-dependent decline of intrinsic axon growth capacity. Recent attempts to augment the growth potential of mature neurons by regulating transcriptional [13–16] or translational machineries [17, 18] have produced encouraging results. For successful axon regeneration, gene expression (Figure 1B) must harmonize with cytoskeletal dynamics (Figure 1C) to appropriately redistribute signaling molecules and

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efficiently reassemble structural components. The mechanisms that control remodeling of the cytoskeletal components and formation of an advancing growth cone after nerve injury will be of particular importance as these processes will directly impact the response to inhibitory molecules, the speed and extent of axon regeneration, and target innervation. Interventions aimed at promoting axon regeneration will eventually converge on cytoskeletal remodeling, but in this review, we discuss the potential and progress in enhancing axon regeneration by directly targeting cytoskeletal components in the growth cone.

Growth cone microtubules are potential targets for promoting axon regeneration

Axon growth is driven by the forward movement of a growth cone, a specialized sensory-motile structure located at the tip of a growing neurite. A growth cone is composed of a central domain rich in microtubules and a peripheral domain enriched in actin filaments. In accordance with the peripheral localization, actin cytoskeleton plays a major part in pathfinding decisions in response to extrinsic cues. Microtubules, on the other hand, are the building blocks of an axon, and thus axon extension occurs through microtubule assembly. Beyond their role as structural scaffolds, continuous remodeling of microtubules is vital for axon growth and guidance [19–22]. The inherent dynamicity enables microtubules to constantly explore the intracellular space, actively interact with signaling molecules, and rapidly undergo reorganization in response to changes. Moreover, growth cone microtubules distribute asymmetrically prior to making pathfinding decisions, and stabilization or destabilization of microtubules on one side of the growth cone is sufficient to induce attractive or repulsive turning [19], suggesting that microtubules play an instructive role in the regulation of growth cone motility and subsequent axon growth.

After injury, it is crucial to reseal the damaged membrane and transform an axonal segment into a motile growth cone that can grow through the hostile local environment. As opposed to advancing growth cones, axons that fail to regenerate form retraction bulbs, structures which are often recognized as hallmarks of regeneration failure [2]. Major axon growth impediments in the CNS, such as myelin-based inhibitors and chondroitin sulfate proteoglycans (CSPGs), induce the formation of dystrophic growth cones and cause massive disorganization of microtubules in the nerve endings [23, 24]. Recently, application of a microtubule-stabilizing drug taxol has been shown to prevent the formation of retraction bulbs [23] and promote axon regeneration after nerve injury [25, 26]. Conversely, destabilization (or disorganization) of microtubules by nocodazole converted growth cones into retraction bulb-like structures and inhibited axon growth [23], suggesting microtubule stability and dynamics as key determinants for the reconstruction of proper nerve endings and subsequent axon regeneration.

There are several potential ways to manipulate microtubule assembly and dynamics during axon regeneration (Box 1). A plethora of microtubule-targeting agents are available that alter microtubule stability and dynamics in diverse ways [27, 28](Glossary). Several endogenous microtubule-regulatory mechanisms can also be manipulated to control microtubule dynamics and function. Notably, there is a battery of microtubule-interacting proteins expressed in the growth cone that associates with the surfaces or ends of microtubules in a highly specific and reversible manner. Manipulation of the individual players that interact with and regulate microtubules might allow precise control over microtubule dynamics and function. Regulating post-translational modifications in microtubule-interacting proteins as well as in microtubules themselves might provide an additional layer of control to fine-tune microtubule function. Given the intricate bidirectional interplay between the actin and microtubule cytoskeletal systems (Box 1),

mechanisms that regulate actin dynamics offer points for further intervention to control microtubule organization and growth cone motility. Considering the dynamically changing environment of the injured CNS, the requirement of expeditious axon extension after nerve injury, and the flexibility and manipulability of microtubules, manipulation of microtubules and growth cone dynamics might represent an attractive strategy towards gaining exquisite control over the speed and trajectory of regenerating axons.

Box 1

Growth cone cytoskeletal machinery

Actin filaments and microtubules are two major cytoskeletal elements that support growth cone-mediate axon assembly. Actin filaments are enriched in the peripheral domain, and are organized into F-actin bundles (aligned or cross-linked linear arrays), F-actin meshwork (a branched dendritic network), or actin arcs. Microtubules are located in the central domain of the growth cone, from which they protrude into the actin-rich peripheral domain and interact with actin filaments. Microtubules are assembled from heterodimers of two tubulin subtypes (six α -tubulin and seven β -tubulin in humans) in a polarized manner, with polymerization occurring mostly on one end of the microtubules (the plus end). At the plus ends, microtubules also undergo cycles of growing and shortening, a process called dynamic instability. Axon extension is ultimately achieved through microtubule assembly in the growth cone, which is subjected to dynamic and specific regulation by multiple factors, including microtubule targeting agents, microtubule-interacting proteins, post-translational modification, and dynamics of actin filaments (Figure I).

Direct stabilization of microtubules by taxol enhances axon regeneration

A recent study showed that taxol could enhance axon regeneration and improve function in a rodent model of spinal cord injury [25], highlighting the therapeutic potential of targeting microtubules to promote axon regeneration. Continuous delivery of taxol at the injury site could reduce fibrotic scarring, in part by preventing transforming growth factor (TGF)- β signaling [25]. TGF- β also has a well-established role in the formation of an astroglial scar by increasing the production and secretion of CSPGs [2]. Thus, the axon growth promoting effect of taxol appears to occur through reducing scar formation in general, including both fibrotic and astroglial scars. Providing neurons with a more supportive environment will clearly aid axon regeneration, but it should be noted that reconstruction of an advancing growth cone is a prerequisite process for subsequent axon regeneration. Notably, taxol promoted axon growth in cultured neurons [25], and induced the formation of growth cone-like structures after nerve injury *in vivo* [23, 25], demonstrating its direct effect on neuronal microtubules. Thus, the enhanced axon regeneration observed *in vivo* might be attributed to both environmental and neuron-intrinsic mechanisms.

A similar conclusion can be reached from another study that applied taxol directly around the lesion site after optic nerve injury [26]. Taxol improved axon regeneration when combined with lens injury, a well-established protocol to augment the intrinsic growth state [26]. In culture, taxol prevented growth cone collapse of retinal ganglion neurons and alleviated axon growth inhibition induced by myelin inhibitors. In an *in vivo* setting, local administration (but not intravitreal injection) of taxol promoted axon regeneration, pointing to the involvement of neuronal microtubules at the lesion site. This study, however, also showed that taxol treatment delayed scar formation and reduced macrophage infiltration into the lesion site [26]. Therefore, as in the spinal cord injury model [25], it is likely that the enhanced axon regeneration after optic nerve crush [26] also results from the combined

effects of taxol on growth cone microtubules and the local environment. With the advent of appropriate genetic tools that allow cell-type-specific control of candidate players, future studies might be able to distinguish the direct role of neuronal microtubules versus microtubules in non-neuronal cells in the local environment during axon regeneration.

Regulating microtubule-interacting proteins to promote axon regeneration

Microtubule dynamics are regulated by a network of microtubule-binding proteins (MBPs). The interaction between MBPs and microtubules is tightly controlled, and phosphorylation is a recurrent theme for such regulation. Glycogen synthase kinase-3 (GSK-3) is known to control several aspects of microtubule dynamics and assembly by phosphorylating multiple MBPs [29, 30]. In a rodent model of spinal cord injury, systemic application of GSK-3 inhibitors could promote axon regeneration and improve function [31]. Although the underlying mechanisms are unknown, it is plausible to speculate that MBPs mediate the axon growth promotion. Collapsin response mediator protein-2 (CRMP-2) is one of the possible candidates for mediating this as it enhances microtubule assembly by binding to tubulin dimers upon GSK-3 inhibition [32, 33]. Moreover, CRMP-2 accumulates in regenerating nerves and its overexpression enhances axon regeneration of injured motor neurons *in vivo* [34], suggesting that CRMP-2 might have promoted axon regeneration downstream of GSK-3. Other MBPs, such as microtubule-associated protein-1B (MAP1-1B) and Tau (substrates of GSK-3 which regulate developmental axon growth [35]), might have played a part, but their roles in axon regeneration are currently unknown.

Mitogen-activated protein kinases (MAPKs) also control the phosphorylation status and activities of several MBPs and are implicated in the regulation of axon regeneration. c-Jun N-terminal kinases (JNKs) are activated following nerve injury and play a role in axon regeneration, in part by activating transcription factors, such as c-Jun and activating transcription factor-3 [36–38]. In addition to the well-established control of nuclear events, recent evidence suggests that JNK regulates local cytoskeletal dynamics. Local application of JNK inhibitors to growth cones impaired growth cone advancement and axon growth within minutes [37, 38], and many of JNK substrates are MBPs that control microtubule dynamics and stability in the growth cone [37, 39–43]. The contribution of JNK substrates to regeneration remains to be investigated, but it is interesting to note that the expression of stathmin family protein-2 (STMN-2), a substrate of JNK, is associated with axon regeneration and is strongly induced following sciatic nerve crush [44, 45]. The exact roles of STMN-2 or other JNK substrates and their interaction with JNK during axon regeneration are yet to be elucidated.

A forward genetic screen in *C. elegans* has identified dual leucine zipper kinase-1 (DLK-1), a MAP-kinase-kinase-kinase (MAPKKK), as an essential regulator of growth cone formation during axon regeneration [46, 47], and coordinate activation of JNK and p38 MAPK-3 pathways has been postulated as downstream signaling of DLK-1 [46, 48]. Interestingly, the DLK/p38 pathway has been shown to coordinate growth cone formation and consolidation of the adjacent axon shaft by regulating local microtubule dynamics in developing neurons [49]. Moreover, growth cone formation and axon growth were impaired by mutating *Phr1* [*PAM (protein associated with Myc)-highwire-RPM-1 (regulator of presynaptic morphology-1)*], an E3-ubiquitin ligase that controls the expression of DLK [49–51], and these defects could be corrected by inhibiting p38 activity or by directly regulating microtubule dynamics [49, 52]. Thus, it is plausible that activation of the DLK/p38 pathway in the distal axon contributes to enhance axon regeneration by controlling local microtubule dynamics.

Microtubule plus end-tracking proteins (+TIPs) comprise a diverse group of proteins that specifically recognize the growing ends of microtubules [53, 54]. Among +TIPs,

adenomatous polyposis coli (APC) [55] and cytoplasmic linker proteins (CLIPs) [56] have been shown to promote neurite extension in developing neurons. A recent study shows that another member of +TIP, CLIP-associated protein (CLASP) is enriched in the growth cone and supports axon regeneration by stabilizing the rapidly growing ends of axonal microtubules downstream of GSK-3 [57]. Interestingly, activities of other +TIPs, such as APC and ATP-dependent chromatin assembly and remodeling factor-7 (ACF-7), are also regulated by GSK-3-dependent phosphorylation [55, 58, 59], suggesting that the enhanced axon regeneration induced by inhibition of GSK-3 (discussed above) [31] might be mediated by +TIPs. The specific accumulation of +TIPs at microtubule plus ends places them in a strategic position to control several aspects of microtubule dynamics during axon regeneration, but the roles of +TIPs and their regulation remain largely unexplored.

Motor proteins comprise another important class of MBPs that are indispensable for microtubule function. Blocking kinesin-5 (also known as kinesin spindle protein, kif11 or Eg5) activity could facilitate axon growth [60–62] and promote microtubule protrusion towards the growth cone periphery [62]. Furthermore, small-molecule inhibitors of kinesin-5 enhanced growth cone invasion into CSPG-coated stripes in a culture assay [60], suggesting the possibility of applying kinesin-5 inhibitors to promote axon regeneration. Given the pivotal roles of motor proteins in growth cone dynamics, understanding motor protein function, direction of movement, and transport and force-generating mechanisms might lead to further insights into possible therapeutic strategies for regeneration.

Recent pharmacological [63] and genetic screening [64] approaches have identified novel molecules that can regulate axon regeneration. One study reports a novel compound that could promote axon regeneration by performing an un-biased screening of a chemical library comprising tri-substituted triazines, with one substituent being a triethylene glycol linker and the other two substituents varying [63]. Although the molecular target(s) of the drug and the mechanisms by which the compound promoted axon regeneration are unknown, it is interesting to note that microtubule dynamics were altered in the growth cone of cultured hippocampal neurons treated with this drug. In another study, a forward genetic screening for intrinsic regulators of axon regeneration identified exchange factor for Arf-6 (EFA-6) [64], a molecule which controls the length and dynamics of microtubules [65]. It remains to be determined if microtubules are direct targets of EFA-6 and how the changes in microtubule dynamics relate to axon regeneration. Nevertheless, these results reiterate the importance of microtubule dynamics in axon regeneration.

Post-translational modification of microtubules –possible role of histone deacetylases (HDACs)

Acetylation is a reversible post-translational modification that has a fundamental role in the epigenetic regulation of gene expression. Histones were the first proteins to be identified as targets of acetylation, and thus, enzymes that mediate acetylation and deacetylation were named histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively [66, 67]. It is now appreciated that the regulatory scope of acetylation is much broader than originally envisioned [68], encompassing chromatin remodeling and transcriptional regulation, microtubule dynamics, metabolism, and longevity [67, 69, 70].

Inhibition of HDAC-6 has been shown to provide neuroprotection and enhance axon growth in cultured neurons that were grown in the presence of inhibitory substrates (ie. myelin-associated proteins and CSPGs) [71]. Notably, axon growth was promoted in a transcription-independent fashion, and local administration of HDAC-6 inhibitors to distal axons was sufficient to overcome CSPG inhibition [71]. Given that α -tubulin is a well-known substrate of HDAC-6 [69] and that tubulin acetylation affects microtubule dynamics and function [69, 72, 73], it is tempting to speculate that increased tubulin acetylation led to the promotion of

axon growth by altering microtubule-binding affinities and activities of many of its binding partners. In addition to microtubule-based mechanisms, actin-dependent processes might have also played a part, as HDAC-6 acetylates several F-actin-binding proteins [68, 74]. Although the exact molecular mechanisms remain to be elucidated, it is plausible that the enhanced axon growth results from the concerted actions of a range of HDAC-6 substrates that regulate microtubule and actin dynamics.

In addition to acetylation, the impacts of other post-translational modifications in tubulin, such as polyglutamylation, polyglycylation, and detyrosination/tyrosination, are beginning to be appreciated ([75] and reviewed in [72]). Although much work remains to be done, the combinatorial use of multiple post-translational modifications in tubulin and microtubule-interacting proteins appears to be one of the likely mechanisms used to generate the versatility and specificity that is required for the control of microtubule dynamics during axon regeneration.

Regulation of actin-based cytoskeletal components

The actin-based cytoskeletal system presents another important component that is vital in the control of microtubule dynamics and growth cone motility (Box 1). Filopodial actin bundles guide microtubule assembly into the growth cone periphery, whereas retrograde flow of actin powered by myosin II carries microtubules rearward, thus posing a dynamic barrier to microtubule advance [76–78]. Blocking retrograde actin flow by inhibiting myosin II activity markedly promoted microtubule extension towards the growth cone periphery on both permissive and inhibitory substrates, including potent growth impediments in the CNS [24, 76]. Importantly, upon myosin II inhibition, CSPG-induced disorganization of growth cone microtubules could be completely prevented, which was accompanied by robust axon assembly [24]. These results suggest that actin-based cytoskeletal components can also be effectively modulated to accelerate axon growth.

After injury, damaged axons encounter an increasing gradient of inhibitory molecules, with the highest concentration of impediments in the lesion epicenter. Thus, axon regeneration strategies must contemplate interventions that can trigger severed axons to grow into the inhibitory terrain, along with methods to accelerate the rate of axon extension (Figure 2). This issue has been addressed by a recent study that used two-compartment chamber platforms to create a laminin (permissive substrate)-CSPG (inhibitory substrate) border [24]. When axons faced the permissive-inhibitory boundary, axons formed retraction bulb-like swellings at the border and failed to enter the axonal compartment, reminiscent of the dystrophic endings stalled at the lesion site *in vivo* [24, 79]. Importantly, local blockade of myosin II activity in distal axons was sufficient to trigger growth cone invasion across the border and migration into the CSPG-coated inhibitory terrain [24]. Thus, targeting myosin II could accelerate axon growth rate on inhibitory substrates and promote growth cone invasion [24]. This suggests that there might be a rather simple strategy to tackle the two distinct challenges (Figure 2) by manipulating the appropriate players. Future experiments will address if these results are recapitulated *in vivo*, but given the prominent role of myosin II in growth cone motility, cell migration and myelination [80–82], it is likely that inhibiting myosin II also promotes axon regeneration *in vivo*.

Opportunities and challenges of microtubule-based approaches

Microtubules have been among the most successful targets in chemotherapy, and a large number of microtubule-targeting agents are in various stages of clinical development for the treatment of a wide range of malignancies [27, 28]. Recent demonstrations that axon regeneration could be enhanced by application of taxol [25, 26] - a clinically established

drug - suggest the exciting possibility of exploiting the growing armamentarium of microtubule-targeting agents to the treatment of CNS repair.

In addition to microtubule-targeting agents, there are a number of small-molecule inhibitors that indirectly alter microtubule dynamics by regulating molecules other than tubulin (Table 1). In this aspect, small-molecule inhibitors of GSK-3 and HDACs are of particular interest. Perturbations in GSK-3 signaling and imbalances in protein acetylation are associated with a broad range of human diseases, such as diabetes, cancer, and a number of neurological disorders [29, 66, 83–87]. Small-molecule inhibitors of GSK-3 or HDACs –which may correct transcriptional and/or cytoskeletal dysfunctions– have proven beneficial in several disease models and are in various stages of clinical development [66, 84–87]. The list of small-molecule inhibitors also includes those that target kinesin family of proteins [88] and actin dynamics, such as inhibitors of myosin II, actin-related protein (Arp) 2/3, and cortactin, all of which have the potential to affect axon regeneration by controlling growth cone dynamics. This rich (and continually growing) resource of small-molecule compounds might offer methods to selectively manipulate microtubule and actin dynamics for a desired outcome. However, because these small-molecule inhibitors regulate many other fundamental cellular processes besides microtubule dynamics, a more thorough examination of the mechanisms of action of the compounds is warranted before using them to promoting axon regeneration, in order to avoid potential unwanted side-effects.

One obvious concern of applying microtubule-targeting agents in the CNS is the frequent occurrence of neurotoxicity [89]. However, in the case of taxol, the effective concentration for enhancing axon regeneration was much lower than the dose used in chemotherapy [25], suggesting that there might be a therapeutic window that can yield beneficial outcomes.

Aside from drug toxicity, the involvement of microtubule dynamics in the plethora of cellular activities raises a fundamental caveat of microtubule-based approaches related to specificity. At least in some instances, however, indications are that alterations in multiple cellular processes actually produce additive or synergistic outcomes, thereby improving therapeutic benefit. As discussed above, taxol might have promoted axon regeneration by stabilizing axonal microtubules, decreasing scar formation, and reducing macrophage infiltration [25, 26]. Inhibition of myosin II might also produce synergy by reorganizing growth cone cytoskeletal components [24] and enhancing myelination in the CNS [90]. Inhibition of HDAC6 also appears to be effective in both enhancing cell viability and axon growth [71]. Moreover, the pan-HDAC inhibitor trichostatin A could reduce spinal cord inflammation, demyelination, and axonal loss [91]. Therefore, targeting microtubule dynamics might offer a way to simultaneously control several processes to create synergism. However, it should also be kept in mind that the involvement of microtubules in multifarious cellular activities may also limit the widespread application of such an approach, unless possible side-effects are thoroughly considered. Therefore, to yield beneficial outcomes, it will be vital to advance our understanding of the mechanisms by which microtubules control various aspects of the regeneration and repair processes in the nervous system.

The major challenge in CNS repair is to induce re-integration of regenerating axons into the neural circuitry and compensate for the functional loss. Axon regeneration achieved through direct manipulation of microtubules may not faithfully recapitulate the original circuitry: As opposed to the highly fasciculated and directed growth of nerve fibers in the intact nervous system, regenerating fibers often grow through ectopic pathways [25] and might form synapses that normally do not exist. The formation of inappropriate connections can create deleterious outcomes, such as neuropathic pain and sensory dysfunction [92]. Therefore, strategies to dictate the direction and connectivity of regenerating fibers should be combined with methods to accelerate axon regeneration.

Concluding remarks and future perspectives

Selective regulation of growth cone dynamics holds much promise as a novel strategy for promoting axon regeneration, but which of the many regulators should be targeted and exactly how such regulation will impact axon regeneration warrant further investigation. Our knowledge of axon growth mostly stems from studies of neuritogenesis during development, when intense and rapid axon growth takes place. The study of developmental axon growth and guidance will continue to reveal fundamental mechanisms that may translate to our understanding of axon regeneration in the adult nervous system. Much work, however, remains to be done to understand to what extent the mechanisms that govern development are conserved in axon regeneration. Given that regenerating axons face distinct hurdles and challenges, re-wiring of the compromised nervous system in mature animals might not be a simple recapitulation of development [93]. Thus, one of the major challenges is to establish appropriate experimental regimes that enable future efforts to define molecular and cellular pathways that are genuinely relevant to axon regeneration. In this regard, the currently available genetic tools might provide limited insights. On the one hand, because microtubules are indispensable for multifarious cellular processes such as cell division, migration, neurite outgrowth, etc. – disruption of microtubule dynamics might produce severe phenotypes before adulthood, precluding further analysis of the genes or pathways that control microtubule dynamics in the context of injury and regeneration in the mature nervous system. On the other hand, even if no obvious phenotypes are detected until adulthood, it will be difficult to entirely rule out compensatory responses that might have taken place during development when interpreting the injury or regenerative responses. Therefore, it is imperative to develop adequate tools that can provide precise temporal control over a gene(s) or pathway(s) of interest specifically during axon regeneration. These tools combined with imaging techniques will help assign specific roles to the players involved in the repair process.

The extent of axon regeneration might be determined by an arsenal of microtubule-regulating factors that are operating at the time of injury and during the repair process. Parameters of microtubule dynamics –such as rates of microtubule growth and shrinkage and the frequency of transitions between catastrophe and rescue– might be controlled by a network of microtubule-interacting molecules tasked with distinct functions to specifically regulate different aspects of axonal growth. Exquisite spatiotemporal regulation of microtubules might be achieved by coordinating the changes in the polymers themselves and their regulators. Furthermore, there is an expanding list of small-molecule compounds that directly or indirectly alter microtubule dynamics, which have the potential to be applied to promote axon regeneration.

Promoting nerve regeneration after traumatic CNS injury appears to be a daunting task, but with continued efforts to bridge together critical components and pathways, a more comprehensive picture will emerge in the future. To yield detectable benefits in the complex and variable arena of human injury, it will be important to consider diverse aspects of the regeneration process and incorporate mechanisms that can protect neurons from noxious stimuli, fuel and accelerate axon growth, improve target innervation, and alleviate inhibitory influences from the environment. In this aspect, microtubule-based approaches are emerging as attractive strategies owing to their potential to regulate multiple cellular activities (Figure 3), and are likely to produce synergy when combined with other interventions. Particular attention to growth cone dynamics will inform the rational design of novel and effective strategies aimed at combating CNS injury and promoting axon regeneration.

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Glossary

Microtubule plus end	Microtubules are polymers of α - and β -tubulin dimers with head-to-tail arrangement. The plus end, where β -tubulin is exposed, is the fast-growing end <i>in vitro</i> and the only end that grows in cells. Microtubule plus ends undergo cycles of rapid growth and shrinkage through the addition or removal of tubulin, a property known as dynamic instability
Microtubule-targeting agents	Microtubule-targeting agents comprise a large number of chemically diverse substances that bind to distinct sites of microtubules (or soluble tubulin) and interfere with the normal functions of microtubules. Microtubule-targeting agents are often classified into two main groups, microtubule stabilizers (e.g. taxanes, such as taxol) and destabilizers (e.g. vinca alkaloids, such as vinblastine), according to their effects on microtubule polymer mass at high concentrations. However, at low concentrations, both classes of compounds suppress microtubule dynamics without changing the polymer mass. They are widely used as chemotherapeutic agents for the treatment of a wide variety of human cancers

References

1. Filbin MT. Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. *Nat Rev Neurosci.* 2003; 4:703–713. [PubMed: 12951563]
2. Silver J, Miller JH. Regeneration beyond the glial scar. *Nat Rev Neurosci.* 2004; 5:146–156. [PubMed: 14735117]
3. Yiu G, He Z. Glial inhibition of CNS axon regeneration. *Nat Rev Neurosci.* 2006; 7:617–627. [PubMed: 16858390]
4. Atwal JK, et al. PirB is a functional receptor for myelin inhibitors of axonal regeneration. *Science.* 2008; 322:967–970. [PubMed: 18988857]
5. Shen Y, et al. PTPsigma is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration. *Science.* 2009; 326:592–596. [PubMed: 19833921]
6. Cafferty WB, et al. MAG and OMgp synergize with Nogo-A to restrict axonal growth and neurological recovery after spinal cord trauma. *J Neurosci.* 2010; 30:6825–6837. [PubMed: 20484625]
7. Kim JE, et al. Nogo-66 receptor prevents raphespinal and rubrospinal axon regeneration and limits functional recovery from spinal cord injury. *Neuron.* 2004; 44:439–451. [PubMed: 15504325]
8. Silver J. Much Ado about Nogo. *Neuron.* 2010; 66:619–621. [PubMed: 20547119]
9. Lee JK, et al. Assessing spinal axon regeneration and sprouting in Nogo-, MAG-, and OMgp-deficient mice. *Neuron.* 2010; 66:663–670. [PubMed: 20547125]
10. Nakamura Y, et al. Paired immunoglobulin-like receptor B knockout does not enhance axonal regeneration or locomotor recovery after spinal cord injury. *J Biol Chem.* 2011; 286:1876–1883. [PubMed: 21087927]
11. Steward O, et al. A re-assessment of the effects of a Nogo-66 receptor antagonist on regenerative growth of axons and locomotor recovery after spinal cord injury in mice. *Exp Neurol.* 2008; 209:446–468. [PubMed: 18234196]

12. Zheng B, et al. Genetic deletion of the Nogo receptor does not reduce neurite inhibition in vitro or promote corticospinal tract regeneration in vivo. *Proc Natl Acad Sci USA*. 2005; 102:1205–1210. [PubMed: 15647357]
13. Moore DL, et al. KLF family members regulate intrinsic axon regeneration ability. *Science*. 2009; 326:298–301. [PubMed: 19815778]
14. Raivich G, et al. The AP-1 transcription factor c-Jun is required for efficient axonal regeneration. *Neuron*. 2004; 43:57–67. [PubMed: 15233917]
15. Seijffers R, et al. ATF3 increases the intrinsic growth state of DRG neurons to enhance peripheral nerve regeneration. *J Neurosci*. 2007; 27:7911–7920. [PubMed: 17652582]
16. Zou H, et al. Axotomy-induced Smad1 activation promotes axonal growth in adult sensory neurons. *J Neurosci*. 2009; 29:7116–7123. [PubMed: 19494134]
17. Liu K, et al. PTEN deletion enhances the regenerative ability of adult corticospinal neurons. *Nat Neurosci*. 2010; 13:1075–1081. [PubMed: 20694004]
18. Park KK, et al. Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. *Science*. 2008; 322:963–966. [PubMed: 18988856]
19. Conde C, Caceres A. Microtubule assembly, organization and dynamics in axons and dendrites. *Nat Rev Neurosci*. 2009; 10:319–332. [PubMed: 19377501]
20. Dent EW, Gertler FB. Cytoskeletal dynamics and transport in growth cone motility and axon guidance. *Neuron*. 2003; 40:209–227. [PubMed: 14556705]
21. Tanaka E, et al. The role of microtubule dynamics in growth cone motility and axonal growth. *J Cell Biol*. 1995; 128:139–155. [PubMed: 7822411]
22. Tanaka EM, Kirschner MW. Microtubule behavior in the growth cones of living neurons during axon elongation. *J Cell Biol*. 1991; 115:345–363. [PubMed: 1918145]
23. Erturk A, et al. Disorganized microtubules underlie the formation of retraction bulbs and the failure of axonal regeneration. *J Neurosci*. 2007; 27:9169–9180. [PubMed: 17715353]
24. Hur EM, et al. Engineering neuronal growth cones to promote axon regeneration over inhibitory molecules. *Proc Natl Acad Sci USA*. 2011; 108:5057–5062. [PubMed: 21383151]
25. Hellal F, et al. Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury. *Science*. 2011; 331:928–931. [PubMed: 21273450]
26. Sengottuvel V, et al. Taxol facilitates axon regeneration in the mature CNS. *J Neurosci*. 2011; 31:2688–2699. [PubMed: 21325537]
27. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. *Nat Rev Cancer*. 2004; 4:253–265. [PubMed: 15057285]
28. Dumontet C, Jordan MA. Microtubule-binding agents: a dynamic field of cancer therapeutics. *Nat Rev Drug Discov*. 2010; 9:790–803. [PubMed: 20885410]
29. Hur EM, Zhou FQ. GSK3 signalling in neural development. *Nat Rev Neurosci*. 2010; 11:539–551. [PubMed: 20648061]
30. Zhou FQ, Snider WD. Cell biology. GSK-3 β and microtubule assembly in axons. *Science*. 2005; 308:211–214.
31. Dill J, et al. Inactivation of glycogen synthase kinase 3 promotes axonal growth and recovery in the CNS. *J Neurosci*. 2008; 28:8914–8928. [PubMed: 18768685]
32. Yoshimura T, et al. GSK-3 β regulates phosphorylation of CRMP-2 and neuronal polarity. *Cell*. 2005; 120:137–149. [PubMed: 15652488]
33. Fukata Y, et al. CRMP-2 binds to tubulin heterodimers to promote microtubule assembly. *Nat Cell Biol*. 2002; 4:583–591. [PubMed: 12134159]
34. Suzuki Y, et al. Collapsin response mediator protein-2 accelerates axon regeneration of nerve-injured motor neurons of rat. *J Neurochem*. 2003; 86:1042–1050. [PubMed: 12887701]
35. Takei Y, et al. Defects in axonal elongation and neuronal migration in mice with disrupted tau and map1b genes. *J Cell Biol*. 2000; 150:989–1000. [PubMed: 10973990]
36. Cavalli V, et al. Sunday Driver links axonal transport to damage signaling. *J Cell Biol*. 2005; 168:775–787. [PubMed: 15738268]
37. Barnat M, et al. Distinct roles of c-Jun N-terminal kinase isoforms in neurite initiation and elongation during axonal regeneration. *J Neurosci*. 2010; 30:7804–7816. [PubMed: 20534829]

38. Lindwall C, Kanje M. 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.* 2005; 29:269–282. [PubMed: 15911351]
39. Chang L, et al. JNK1 is required for maintenance of neuronal microtubules and controls phosphorylation of microtubule-associated proteins. *Dev Cell.* 2003; 4:521–533. [PubMed: 12689591]
40. Gdalyahu A, et al. DCX, a new mediator of the JNK pathway. *EMBO J.* 2004; 23:823–832. [PubMed: 14765123]
41. Goedert M, et al. Phosphorylation of microtubule-associated protein tau by stress-activated protein kinases. *FEBS Lett.* 1997; 409:57–62. [PubMed: 9199504]
42. Reynolds CH, et al. Stress-activated protein kinase/c-jun N-terminal kinase phosphorylates tau protein. *J Neurochem.* 1997; 68:1736–1744. [PubMed: 9084448]
43. Tararuk T, et al. JNK1 phosphorylation of SCG10 determines microtubule dynamics and axodendritic length. *J Cell Biol.* 2006; 173:265–277. [PubMed: 16618812]
44. Mason MR, et al. Transcriptional upregulation of SCG10 and CAP-23 is correlated with regeneration of the axons of peripheral and central neurons in vivo. *Mol Cell Neurosci.* 2002; 20:595–615. [PubMed: 12213442]
45. Voria I, et al. Improved sciatic nerve regeneration by local thyroid hormone treatment in adult rat is accompanied by increased expression of SCG10. *Exp Neurol.* 2006; 197:258–267. [PubMed: 16289052]
46. Hammarlund M, et al. Axon regeneration requires a conserved MAP kinase pathway. *Science.* 2009; 323:802–806. [PubMed: 19164707]
47. Yan D, et al. The DLK-1 kinase promotes mRNA stability and local translation in *C. elegans* synapses and axon regeneration. *Cell.* 2009; 138:1005–1018. [PubMed: 19737525]
48. Nix P, et al. Axon regeneration requires coordinate activation of p38 and JNK MAPK pathways. *Proc Natl Acad Sci USA.* 2011; 108:10738–10743. [PubMed: 21670305]
49. Lewcock JW, et al. The ubiquitin ligase Phr1 regulates axon outgrowth through modulation of microtubule dynamics. *Neuron.* 2007; 56:604–620. [PubMed: 18031680]
50. Collins CA, et al. Highwire restrains synaptic growth by attenuating a MAP kinase signal. *Neuron.* 2006; 51:57–69. [PubMed: 16815332]
51. Nakata K, et al. Regulation of a DLK-1 and p38 MAP kinase pathway by the ubiquitin ligase RPM-1 is required for presynaptic development. *Cell.* 2005; 120:407–420. [PubMed: 15707898]
52. Hendricks M, Jesuthasan S. PHR regulates growth cone pausing at intermediate targets through microtubule disassembly. *J Neurosci.* 2009; 29:6593–6598. [PubMed: 19458229]
53. Akhmanova A, Steinmetz MO. Tracking the ends: a dynamic protein network controls the fate of microtubule tips. *Nat Rev Mol Cell Biol.* 2008; 9:309–322. [PubMed: 18322465]
54. Galjart N. Plus-end-tracking proteins and their interactions at microtubule ends. *Curr Biol.* 2010; 20:R528–537. [PubMed: 20620909]
55. Zhou FQ, et al. NGF-induced axon growth is mediated by localized inactivation of GSK-3 β and functions of the microtubule plus end binding protein APC. *Neuron.* 2004; 42:897–912. [PubMed: 15207235]
56. Neukirchen D, Bradke F. Cytoplasmic linker proteins regulate neuronal polarization through microtubule and growth cone dynamics. *J Neurosci.* 2011; 31:1528–1538. [PubMed: 21273437]
57. Hur EM, et al. GSK3 controls axon growth via CLASP-mediated regulation of growth cone microtubules. *Genes Dev.* 2011; 25:1968–1981. [PubMed: 21937714]
58. Kumar P, et al. GSK3 β phosphorylation modulates CLASP-microtubule association and lamella microtubule attachment. *J Cell Biol.* 2009; 184:895–908. [PubMed: 19289791]
59. Wu X, et al. Skin stem cells orchestrate directional migration by regulating microtubule-ACF7 connections through GSK3 β . *Cell.* 2011; 144:341–352. [PubMed: 21295697]
60. Lin S, et al. Inhibition of Kinesin-5, a microtubule-based motor protein, as a strategy for enhancing regeneration of adult axons. *Traffic.* 2011; 12:269–286. [PubMed: 21166743]
61. Myers KA, Baas PW. Kinesin-5 regulates the growth of the axon by acting as a brake on its microtubule array. *J Cell Biol.* 2007; 178:1081–1091. [PubMed: 17846176]

62. Nadar VC, et al. Kinesin-5 is essential for growth-cone turning. *Curr Biol.* 2008; 18:1972–1977. [PubMed: 19084405]
63. Usher LC, et al. A chemical screen identifies novel compounds that overcome glial-mediated inhibition of neuronal regeneration. *J Neurosci.* 2010; 30:4693–4706. [PubMed: 20357120]
64. Chen L, et al. Axon Regeneration Pathways Identified by Systematic Genetic Screening in *C. elegans*. *Neuron.* 2011; 71:1043–1057. [PubMed: 21943602]
65. O'Rourke SM, et al. *Caenorhabditis elegans* EFA-6 limits microtubule growth at the cell cortex. *Nat Cell Biol.* 2010; 12:1235–1241. [PubMed: 21076413]
66. Kazantsev AG, Thompson LM. Therapeutic application of histone deacetylase inhibitors for central nervous system disorders. *Nat Rev Drug Discov.* 2008; 7:854–868. [PubMed: 18827828]
67. Kouzarides T. Chromatin modifications and their function. *Cell.* 2007; 128:693–705. [PubMed: 17320507]
68. Choudhary C, et al. Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science.* 2009; 325:834–840. [PubMed: 19608861]
69. Hubbert C, et al. HDAC6 is a microtubule-associated deacetylase. *Nature.* 2002; 417:455–458. [PubMed: 12024216]
70. Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. *Biochem J.* 2007; 404:1–13. [PubMed: 17447894]
71. Riveccio MA, et al. HDAC6 is a target for protection and regeneration following injury in the nervous system. *Proc Natl Acad Sci USA.* 2009; 106:19599–19604. [PubMed: 19884510]
72. Janke C, Kneussel M. Tubulin post-translational modifications: encoding functions on the neuronal microtubule cytoskeleton. *Trends Neurosci.* 2010; 33:362–372. [PubMed: 20541813]
73. Matsuyama A, et al. In vivo destabilization of dynamic microtubules by HDAC6-mediated deacetylation. *EMBO J.* 2002; 21:6820–6831. [PubMed: 12486003]
74. Zhang X, et al. HDAC6 modulates cell motility by altering the acetylation level of cortactin. *Mol Cell.* 2007; 27:197–213. [PubMed: 17643370]
75. Erck C, et al. A vital role of tubulin-tyrosine-ligase for neuronal organization. *Proc Natl Acad Sci USA.* 2005; 102:7853–7858. [PubMed: 15899979]
76. Burnette DT, et al. Filopodial actin bundles are not necessary for microtubule advance into the peripheral domain of *Aplysia* neuronal growth cones. *Nat Cell Biol.* 2007; 9:1360–1369. [PubMed: 18026092]
77. Medeiros NA, et al. Myosin II functions in actin-bundle turnover in neuronal growth cones. *Nat Cell Biol.* 2006; 8:215–226. [PubMed: 16501565]
78. Schaefer AW, et al. Filopodia and actin arcs guide the assembly and transport of two populations of microtubules with unique dynamic parameters in neuronal growth cones. *J Cell Biol.* 2002; 158:139–152. [PubMed: 12105186]
79. Neumann S, Woolf CJ. Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury. *Neuron.* 1999; 23:83–91. [PubMed: 10402195]
80. Brown ME, Bridgman PC. Myosin function in nervous and sensory systems. *J Neurobiol.* 2004; 58:118–130. [PubMed: 14598375]
81. Diefenbach TJ, et al. Myosin 1c and myosin IIB serve opposing roles in lamellipodial dynamics of the neuronal growth cone. *J Cell Biol.* 2002; 158:1207–1217. [PubMed: 12356865]
82. Vicente-Manzanares M, et al. Non-muscle myosin II takes centre stage in cell adhesion and migration. *Nat Rev Mol Cell Biol.* 2009; 10:778–790. [PubMed: 19851336]
83. Christensen DP, et al. Histone deacetylase (HDAC) inhibition as a novel treatment for diabetes mellitus. *Mol Med.* 2011; 17:378–390. [PubMed: 21274504]
84. Chuang DM, et al. Multiple roles of HDAC inhibition in neurodegenerative conditions. *Trends Neurosci.* 2009; 32:591–601. [PubMed: 19775759]
85. Jope RS, Johnson GV. The glamour and gloom of glycogen synthase kinase-3. *Trends Biochem Sci.* 2004; 29:95–102. [PubMed: 15102436]
86. Minucci S, Pelicci PG. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat Rev Cancer.* 2006; 6:38–51. [PubMed: 16397526]

87. Rayasam GV, et al. Glycogen synthase kinase 3: more than a namesake. *Br J Pharmacol.* 2009; 156:885–898. [PubMed: 19366350]
88. Huszar D, et al. Kinesin motor proteins as targets for cancer therapy. *Cancer Metastasis Rev.* 2009; 28:197–208. [PubMed: 19156502]
89. Canta A, et al. Tubulin: a target for antineoplastic drugs into the cancer cells but also in the peripheral nervous system. *Curr Med Chem.* 2009; 16:1315–1324. [PubMed: 19355888]
90. Wang H, et al. Myosin II has distinct functions in PNS and CNS myelin sheath formation. *J Cell Biol.* 2008; 182:1171–1184. [PubMed: 18794332]
91. Camelo S, et al. Transcriptional therapy with the histone deacetylase inhibitor trichostatin A ameliorates experimental autoimmune encephalomyelitis. *J Neuroimmunol.* 2005; 164:10–21. [PubMed: 15885809]
92. Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. *Lancet.* 1999; 353:1959–1964. [PubMed: 10371588]
93. Gabel CV, et al. Distinct cellular and molecular mechanisms mediate initial axon development and adult-stage axon regeneration in *C. elegans*. *Development.* 2008; 135:1129–1136. [PubMed: 18296652]
94. Jiang H, et al. Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3 β and its upstream regulators. *Cell.* 2005; 120:123–135. [PubMed: 15652487]
95. Ryves WJ, Harwood AJ. Lithium inhibits glycogen synthase kinase-3 by competition for magnesium. *Biochem Biophys Res Commun.* 2001; 280:720–725. [PubMed: 11162580]
96. Kim WY, et al. Essential roles for GSK-3 α and GSK-3 β -primed substrates in neurotrophin-induced and hippocampal axon growth. *Neuron.* 2006; 52:981–996. [PubMed: 17178402]
97. Garrido JJ, et al. GSK3 α and GSK3 β are necessary for axon formation. *FEBS Lett.* 2007; 581:1579–1586. [PubMed: 17391670]
98. Shi SH, et al. APC and GSK-3 β are involved in mPar3 targeting to the nascent axon and establishment of neuronal polarity. *Curr Biol.* 2004; 14:2025–2032. [PubMed: 15556865]
99. Tang M, et al. Interactions of Wnt/ β -catenin signaling and sonic hedgehog regulate the neurogenesis of ventral midbrain dopamine neurons. *J Neurosci.* 2010; 30:9280–9291. [PubMed: 20610763]
100. Haggarty SJ, et al. Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. *Proc Natl Acad Sci USA.* 2003; 100:4389–4394. [PubMed: 12677000]
101. Tapia M, et al. Impaired function of HDAC6 slows down axonal growth and interferes with axon initial segment development. *PLoS One.* 2010; 5:e12908. [PubMed: 20886111]
102. Kozikowski AP, et al. Functional differences in epigenetic modulators-superiority of mercaptoacetamide-based histone deacetylase inhibitors relative to hydroxamates in cortical neuron neuroprotection studies. *J Med Chem.* 2007; 50:3054–3061. [PubMed: 17539623]
103. Butler KV, et al. Rational design and simple chemistry yield a superior, neuroprotective HDAC6 inhibitor, tubastatin A. *J Am Chem Soc.* 2010; 132:10842–10846. [PubMed: 20614936]
104. Finnin MS, et al. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature.* 1999; 401:188–193. [PubMed: 10490031]
105. Gaub P, et al. HDAC inhibition promotes neuronal outgrowth and counteracts growth cone collapse through CBP/p300 and P/CAF-dependent p53 acetylation. *Cell Death Differ.* 2010; 17:1392–1408. [PubMed: 20094059]
106. Luthi-Carter R, et al. SIRT2 inhibition achieves neuroprotection by decreasing sterol biosynthesis. *Proc Natl Acad Sci USA.* 2010; 107:7927–7932. [PubMed: 20378838]
107. Outeiro TF, et al. Sirtuin 2 inhibitors rescue α -synuclein-mediated toxicity in models of Parkinson's disease. *Science.* 2007; 317:516–519. [PubMed: 17588900]
108. Falnrikar A, et al. Kinesin-5, a mitotic microtubule-associated motor protein, modulates neuronal migration. *Mol Biol Cell.* 2011; 22:1561–1574. [PubMed: 21411631]
109. Yan Y, et al. Inhibition of a mitotic motor protein: where, how, and conformational consequences. *J Mol Biol.* 2004; 335:547–554. [PubMed: 14672662]

110. Brier S, et al. Identification of the protein binding region of S-trityl-L-cysteine, a new potent inhibitor of the mitotic kinesin Eg5. *Biochemistry*. 2004; 43:13072–13082. [PubMed: 15476401]
111. Luo L, et al. ATP-competitive inhibitors of the mitotic kinesin KSP that function via an allosteric mechanism. *Nat Chem Biol*. 2007; 3:722–726. [PubMed: 17922005]
112. Brown JA, et al. Dorsal root ganglion neurons react to semaphorin 3A application through a biphasic response that requires multiple myosin II isoforms. *Mol Biol Cell*. 2009; 20:1167–1179. [PubMed: 19109430]
113. Gallo G. RhoA-kinase coordinates F-actin organization and myosin II activity during semaphorin-3A-induced axon retraction. *J Cell Sci*. 2006; 119:3413–3423. [PubMed: 16899819]
114. Solecki DJ, et al. Myosin II motors and F-actin dynamics drive the coordinated movement of the centrosome and soma during CNS glial-guided neuronal migration. *Neuron*. 2009; 63:63–80. [PubMed: 19607793]
115. Ostap EM. 2,3-Butanedione monoxime (BDM) as a myosin inhibitor. *J Muscle Res Cell Motil*. 2002; 23:305–308. [PubMed: 12630704]
116. Poulain FE, Sobel A. The microtubule network and neuronal morphogenesis: Dynamic and coordinated orchestration through multiple players. *Mol Cell Neurosci*. 2010; 43:15–32. [PubMed: 19660553]

Figure 1. Possible targets and strategies to promote axon regeneration. (A) Modulation of signaling triggered by extrinsic factors

Modulating signaling pathways that are activated by extrinsic factors represents perhaps the most extensively investigated approach for promoting axon regeneration. These strategies include methods to antagonize inhibitory signaling elicited by a growing list of axon growth impediments, such as chondroitin sulfate proteoglycans (CSPGs), myelin-associated inhibitors, and classical repulsive guidance cues. Alternatively, repair or regeneration strategies aimed at augmenting beneficial signaling, such as growth factor-, cytokine-, or extracellular matrix (ECM) integrin-signaling, are also being investigated in order to enhance cell viability and promote axon extension. **(B) Regulation of the machineries that control gene expression.** Regulators of the transcriptional or translational processes can serve as potential targets to enhance the intrinsic axon regeneration capacity. Recent evidence suggests the possibility of modulating players in the phosphatase and tensin homolog (PTEN)/phosphoinositide 3-kinase (PI3K) pathway to control protein translation mediated by the mammalian target of rapamycin (mTOR) pathway [18]. Transcription factors and cofactors are also possible candidates for promoting axon regeneration [13–15].

These factors might be controlled by injury signals initiated at the lesion site, but the activation/inactivation mechanisms remain largely unknown. Modulating the transcriptional or translational machineries is likely to stimulate the general axon growth potential and aid axon regeneration by altering the expression of a number of regeneration-associated genes and initiating signaling pathways that are required for cell survival and axon assembly. (C) **Direct manipulation of cytoskeletal components.** Interventions that directly control cytoskeletal components, especially in the growth cone, are emerging as promising strategies to enhance axon regeneration. Mechanisms that control actin and microtubule dynamics in the growth cone would have a direct impact on axon regeneration by accelerating the speed of axon extension and affecting the responses of the growth cone to axon growth impediments. Modulation of microtubule dynamics in the growth cone can be achieved by direct post-translational modification of microtubules, or by the regulation of microtubule-interacting proteins, such as classic microtubule-binding proteins (MBPs) and microtubule plus end-binding proteins (+TIPs). Manipulation of actin filaments (actin bundles and actin meshwork) can also affect microtubule organization via dynamic interactions between the two cytoskeletal systems. Microtubule-based motor proteins that control intracellular trafficking might also serve as potential targets for promoting axon regeneration.

Figure 2. Experimental interventions that enhance growth cone invasion and/or axon growth rate

After nerve injury, a damaged axon encounters an increasing gradient of inhibitory molecules, often leading to the formation of a retraction bulb at the lesion site. The first step for successful axon regeneration is the formation of an advancing growth cone that can grow into the inhibitory milieu. This issue has been experimentally tested by designing assays such as stripe assays, differentially coated two-compartment chamber platforms, or other methods to create an inhibitory terrain adjacent to a permissive zone (ie. a permissive-inhibitory border). Recent studies showed that local application of taxol [25], or pharmacological inhibitors of myosin II [24] or kinesin-5 [60] could trigger severed axons to migrate into the inhibitory terrain. After crossing the permissive-inhibitory border, axons face another challenge, which is to promote axonal growth and extension over the inhibitory zone. Axonal growth over inhibitory molecules is usually examined by measuring the lengths of axons or the rate of axon extension from neurons growing on uniformly coated inhibitory molecules or substrates *in vitro*. Application of taxol [25] or inhibition of myosin II [24], kinesin 5 [60, 61], or histone deacetylase-6 (HDAC-6) [71] (achieved either by siRNA-mediated depletion or by applying pharmacological inhibitors) could enhance axon growth. Some pharmacological inhibitors of GSK-3 have also been shown to enhance axon growth over CNS inhibitors [31]. Notably, manipulation of certain cytoskeletal components could promote both growth cone invasion and axon extension. Many strategies aimed at promoting axon regeneration are based on our understanding of the mechanisms that control the rate of axon extension, especially over inhibitory substrates. It remains to be determined if other interventions that accelerate axon growth also have an effect on growth cone invasion.

Figure 3. Challenges posed at the injury site that can be targeted by microtubule-based approaches

Severed neurons face multiple challenges at the injury site. Damaged neurons must form growth cones and assemble axons by overcoming the hostile CNS environment. Furthermore, once the axons are assembled, axons need to be re-myelinated for proper functioning and signaling within the nervous system. Recent studies have shown that axon regeneration *in vivo* could be promoted by direct application of a microtubule-stabilizing drug taxol at the injury site [25, 26]. Reorganization of growth cone microtubule structures were evident in taxol-treated animals [23, 25], and there was a substantial reduction in scar formation [25, 26] and macrophage infiltration [26]. Therefore, the enhanced axon regeneration observed *in vivo* is likely to be attributed to the combined effects of taxol on multiple microtubule-based cellular processes involving both neuronal and non-neuronal responses. It should be noted that local responses at the injury site must be coordinated with activation of the gene expression program in the cell body as well as with axonal transport mechanisms in order to maximize axon regeneration. Together, such effects help the axons to overcome the inhibitory environment at the injury site and undergo long-distance regeneration.

Box 1 Figure I. Regulation of microtubule assembly in the growth cone

Microtubule (MT) dynamics are regulated in large part by an extensive portfolio of (a) MT-interacting proteins that bind to tubulin dimers or assembled MTs [116]. They include classical MBPs that recognize the lattices of MTs and MT plus end-binding proteins (+TIPs) that specifically associate with the growing ends of MTs, both of which function to stabilize polymerized MTs. Conversely, there are proteins that also bind to the plus ends but act specifically to induce MT depolymerization. In addition, MT-severing proteins bind along the MT lattices and function to fragmentize MTs. Lastly, the MT-based motor proteins can transport polymerized MTs (or other cargos) and control MT protrusion in the growth cone. (b) MTs in the growth cone can also be modulated by application of MT-targeting agents that directly affect MT stability and dynamics (c) Post-translational modification of MT-interacting proteins is a well-established mechanism to control their affinities for MTs (or soluble tubulin). MTs themselves are also substrates for a number of post-translational modifications, such as acetylation/deacetylation, tyrosination/detyrosination, polyglutamylation, polyglycylation, etc., which induce structural changes to the polymer and affect the interaction with their binding partners (reviewed in [72]). It is conceivable that combinations of multiple post-translational modifications in MTs and in their binding partners create a framework for the versatility and specificity of MT dynamics and function. (d) Growth cone motility is determined by the intricate bidirectional interplay between actin and MT dynamics. In the growth cone, actin bundles in the filopodial act as tracks along which MTs advance towards growth cone periphery, whereas retrograde flow of actin transports MTs rearward. Therefore, regulation of the organization and/or dynamics of actin filaments can indirectly control MT protrusion in the growth cone.

Table 1

Small-molecule compounds that affect neuronal function by altering microtubule- or actin-binding proteins^{1,4}.

Target molecule	Small-molecule inhibitors ²	Mechanism of action	Tested functions in the nervous system	Effects on axon growth	Refs
GSK-3	Lithium	Competitive inhibitor of Mg ²⁺	Survival, polarity, axon growth	Promote axon regeneration in a rodent model of spinal cord injury ³	[31, 94, 95]
	BIO	Competition with ATP binding sites	Axon growth	Promote/Inhibit ⁴	[96]
	SB216763	Competition with ATP binding sites	Polarity, axon growth	Promote/Inhibit ⁴	[31, 94, 97]
	SB415286	Competition with ATP binding sites	Polarity, axon growth	Promote axon regeneration in a rodent model of spinal cord injury ³	[31, 94, 97]
HDAC-6	AR-A014418	Competition with ATP binding sites	Polarity, axon growth	Inhibit	[97, 98]
	CHIR-99021	Competition with ATP binding sites	Neurogenesis	Unknown	[99]
	Tubacin	Unknown	Axon growth	Inhibit	[100, 101]
	Mercaptoacetamide	Chelate the zinc ion in the active site of HDACs	Survival, axon growth	Promote	[71, 102]
	Tubastatin A	Unknown	Survival	Unknown	[103]
	Trichostatin A (TSA)	Interacting with the catalytic site of HDACs	Axon growth	Promote	[71, 104, 105]
Sirt-2	AGK2	Block the NAD ⁺ site of Sirt2	Neuroprotection in <i>Drosophila</i> model of Parkinson's disease ³	Unknown	[106, 107]
	Compound B2	Unknown	Neuroprotection in <i>Drosophila</i> model of Parkinson's disease ³	Unknown	[107]
	Monastrol	Allosterically inhibits ATPase activity	Axon growth, neuronal migration	Promote	[60, 108, 109]
Kinesin-5	S-Trityl-L-cysteine	Allosterically inhibits ATPase activity	Axon growth	Promote	[60, 110]
	HR22CI6	Unknown	Axon growth	Promote	[60]
	Ispinesib	Allosterically inhibits ATPase activity	Unknown	Unknown	[88, 111]
	Blebbistatin	Non-muscle myosin II ATPase inhibitor	Neuronal migration, axon growth myelination, axon guidance	Promote	[24, 90, 112–114]
Myosin II	2,3-Butanedione monoxime (BDM)	Myosin superfamily ATPase inhibitor	Growth cone motility	Unknown	[115]

¹ It should be noted that many of these compounds have other important cellular functions and mechanisms of action besides those mentioned here.

² Abbreviations: AGK2, 2-Cyano-3-[[5-(2,5-dichlorophenyl)-2-furanyl]-N-5-quinolinyl-2-propenamide; AR-A014418, N-(4-Methoxybenzyl)-N'-(5-nitro-1,3-thiazol-2-yl)urea; AZD4877, N-methyl-2-[[3-[(1E)-2-(2-pyridinyl)ethenyl]-1H-indazol-6-yl]thio]-benzamide; BIO, 6-Bromindirubin-30-oxime; CHIR99021, 6-((2-((4-(2,4-Dichlorophenyl)-5-(4-methyl-1H-imidazol-2-yl)pyrimidin-2-

y)amino)ethyl)amino)nicotinonitrile; SB216763, 3-(2,4-Dichlorophenyl)-4-(1-methyl-1H-3-yl)-1H-pyrrole-2,5-dione; SB 415286, 3-[(3-Chloro-4-hydroxyphenyl)-(2-nitrophenyl)-1H-pyrrol-2,5-dione; Sirt-2, NAD-dependent deacetylase sirtuin-2.

³ Effects of drugs were examined in an in vivo setting.

⁴ Inhibition of GSK-3 has been shown to both promote and inhibit axon growth. To reconcile the controversy, it has been suggested that the extent of GSK-3 inhibition determines the outcome: axon growth is promoted by moderate inhibition of GSK3 activity, whereas it is blocked by strong suppression of GSK-3 [95]. It is likely that distinct substrates are involved in the opposite responses, but further experiments are required to provide an explanation for the discrepancy.