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Spatial and temporal dynamics of neurite regrowth

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Injury to mature neurites triggers a series of events that have both growth promoting and inhibitory roles. Recent evidence from a variety of experimental models has revealed new neuronal regrowth modulators. The action of these modulators must be precisely regulated both in time and space, and involves multiple cellular processes including retrograde signaling and local translation in the injured neurite. New genetic techniques, in combination with pharmacological approaches, have served to advance mechanistic dissection of neuronal response to injury. Better understanding of the spatio-temporal cues would greatly aid in the development of effective regenerative therapies.

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Introduction

Neurite outgrowth following injury is an important step in repairing neuronal damage. The extent of outgrowth is long known to depend on extrinsic and intrinsic signaling pathways [1,2]. Recent research has revealed new signaling pathways that may help increase the intrinsic neuronal growth potential. An insult to neurites generates injury signals that must be relayed to the cell body. Concomitant with this process is the initiation of growth from the injured neurite. Cytoskeletal structure undergoes major remodeling immediately after injury, and persistent growth of neurites is dependent on microtubule dynamics. Regulators of mRNA can play important roles in coordinating local signaling and trafficking of the injury signals. Among the new pathways, the MAPKKK dual-leucine zipper kinase (DLK) has emerged as a master regulator of injury-triggered stress responses. In this minireview, we summarize recent insights into the regulation of microtubule outgrowth during regeneration. We then look into the formation and transport of the immediate response at the injury site and highlight new work that elucidates the mechanism of action of DLK. We conclude this review by discussing the relevance of spatio-temporal dynamics in the neuronal response to injury.

Insights into microtubule remodeling in neurite regrowth

The regenerative failure of CNS neurons post injury has long been attributed to the inability of these axons to generate and maintain growth cone-like structures. Indeed, the formation of dystrophic ends in brain and spinal cord neurons was observed by Cajal a century ago, when he found that transected axons extend for short distances before they stop and form swollen and stunted ends, which he termed 'axon retraction bulbs' [3]. Only recently has the underlying cause been directly linked to the failure in modulating microtubule (MT) dynamics in the injured axon [4,5].

Recent pharmacological therapies have provided strong evidence for enhanced regrowth through modulation of MT dynamics, highlighting the importance of cytoskeletal remodeling following neurite injury. Taxol, an MT stabilizing and anti-cancer drug, can prevent retraction bulb formation and promote axon regeneration in CNS injury models [6•,7•]. Taxol also inhibits glial scar formation, thereby ameliorating the inhibitory environment, which is long known to contribute to CNS regenerative failure [6•]. Caltubin, a novel calcium binding protein isolated in water snails, can bind to β-tubulin in mammalian cells and can prevent axon retraction after injury, potentially by modulating β-tubulin levels at the growth cone [8•]. Caltubin has no clear mammalian homologs, but its effectiveness in promoting axon outgrowth in PC12 cells and mouse cortical neurons suggests potential therapeutic strategy.

An exciting series of recent work has revealed multiple regulatory pathways that modulate MT dynamics in a variety of model organisms. GFP-tagged MT plus-end binding proteins provide valuable tools for *in vivo* visualization of growing MT polymers [9•,10••,11••,12]. Chen *et al.* showed that in *Drosophila* larval sensory neurons the cytoskeletal changes elicited by axonal injury are not restricted to the injured axon [9•]. Severing the axon appears to activate a JNK dependent pathway that increases MT growth in the dendrites, resulting in increased dendritic stability. The increase in the number of growing microtubules in the dendrites requires the MT nucleation protein γ-tubulin, yet regeneration from the

severed axon is normal with reduced levels of γ-tubulin [9•]. In this model, injury triggers two parallel pathways: one to regenerate from the injured neurite and another to protect the remaining neurites from further damage.

Several studies have shown MT dynamics are essential for axon regeneration in *C. elegans* [10••,11••,12]. Loss of an MT plus end binding protein EBP-1 results in the formation of expanded but immotile growth cones and inhibits regrowth of touch neurons [10••]. Axotomy triggers local up-regulation of growing MTs near the injury site, which steadies into a growth phase with reduced catastrophe frequency, correlating with the formation and extension of a growth cone-like structure [11••,13]. Touch neuron axons fail to initiate regeneration in a dominant β-tubulin mutant, although the axons that manage to initiate regeneration do so to the normal extent [12]. These studies highlight the importance of growth cone initiation in axon regeneration and suggest that growth cone initiation and axon extension may require different cytoskeletal regulation.

Microtubules are characterized by their 'dynamic instability', a behavior marked with rapid switches between persistent growth and shrinkage. MT catastrophe factors typically accumulate at the ends of MTs and mediate their depolymerization [14]. Current studies have revealed roles for numerous MT catastrophe factors in neurite regrowth [10••,11••,15]. The N-terminus of EFA-6, a putative MT catastrophe factor, is necessary and sufficient to limit MT growth near the cell cortex of *C. elegans* embryonic cells [16]. Chen *et al.* showed that EFA-6 acts cell autonomously to inhibit regrowth in *C. elegans* touch neurons [10••]. In regrowing axons, overexpression of EFA-6 N-terminus reduces the number of growing MTs, while loss of *efa-6* increases them. The regenerative block caused by EFA-6 overexpression could be overcome by taxol, supporting a primary role of EFA-6 in MT destabilization. *C. elegans* kinesin-13, a MT depolymerizing protein [17], maintains steady state MT number in uninjured touch axons, and inhibits growing MTs after injury [11••]. In *Drosophila* sensory axons, normal gene dosage of MT severing protein Spastin is required for regeneration [15]. Loss of one copy of spastin blocks regeneration, and overexpression significantly reduces regeneration length. The differential activity of these MT destabilizing proteins on neurite regrowth may reflect the timing of their action. Both EFA-6 and Kinesin-13 reduce MT dynamics in early stages of regrowth to inhibit growth cone formation [10••,11••]. On the other hand, spastin is probably required to maintain persistent MT growth after the initial remodeling process [15], perhaps by generating local seeds that act as substrates for new MT polymerization [18] (Figure 1).

Tubulins and MTs have long been known to undergo post-translational modifications (PTMs), which can influence cellular growth and transport properties by regulating MT interactions with MT associated proteins (MAPs) [19,20]. MT-PTMs including Δ2 modification, polyglutamylation, tyrosination, and acetylation, are catalyzed by a variety of enzymes that are widely expressed in vertebrates and invertebrates [21]. Specific MT-PTMs can also affect motor protein affinity and stability in the nervous system. In *C. elegans*, carboxypeptidases CCPP-1 and CCPP-6 [22,23], which catalyze the Δ2 modification of tubulin, act cell autonomously to promote regrowth, while a putative tubulin polyglutamylase TTLL-5 inhibits initial growth cone formation [11••]. Δ2 modifications, which are associated with stabilized MTs, are enriched in the mammalian brain [21,24], suggesting the importance of MT stability in neurites. Tubulin deacetylation and tyrosination, both of which have been associated with unstable microtubules [20], are upregulated in a mouse model of PNS injury [25] (Figure 1). MT detyrosination suppresses kinesin-13 activity *in vitro* [26], and tubulin polyglutamylation promotes spastin mediated MT severing [27]. Taken together, these studies highlight the importance of precise modulation of the levels of tubulin PTMs in enhancing regrowth ability after injury.

Advances in understanding local signals and retrograde trafficking after injury

Since injury generally occurs at a distance from the cell body, a long-standing notion is that generation of injury signals and retrograde transport are necessary for a regenerative response [28,29]. Recent studies provide strong evidence for this model [30–32]. Importins, comprised of α and β subunits, retrogradely transport nuclear localization signal (NLS)-bearing cargo proteins [33,34]. Interestingly, Importin β1 mRNA containing a longer 3′UTR is localized to the sciatic nerve axon [30••]. In response to injury, Importin β1 is locally translated, leading to heterodimer formation with Importin α to facilitate retrograde transport [35]. To specifically address the axonal role of Importin β1 Perry *et al.* created a knockout mouse lacking only the axonal Importin β1 mRNA isoform. These mice showed a decreased response to axon injury and delayed behavioral recovery, which was attributed to the lack of local translation of Importin β1. They also observed decreased transcription of genes that are normally upregulated after injury, supporting a crucial role of axonal Importin β1 for regeneration [30••].

What might be the cargos for retrograde transport? Using previously collected phosphoproteomic and microarray data, Ben-Yakkov *et al.* identified several transcription factors that are retrogradely transported from the axon [36•,37]. They showed that retrograde transport of phosphorylated STAT3 is essential for neuronal survival post sciatic crush injury [36•]. Injecting STAT3 NLS peptides into the sciatic nerve competed with nuclear import of phosphorylated STAT3 and dynein mediated retrograde transport of pSTAT3, resulting in decreased neuronal survival after injury [36•]. Additionally, an independent