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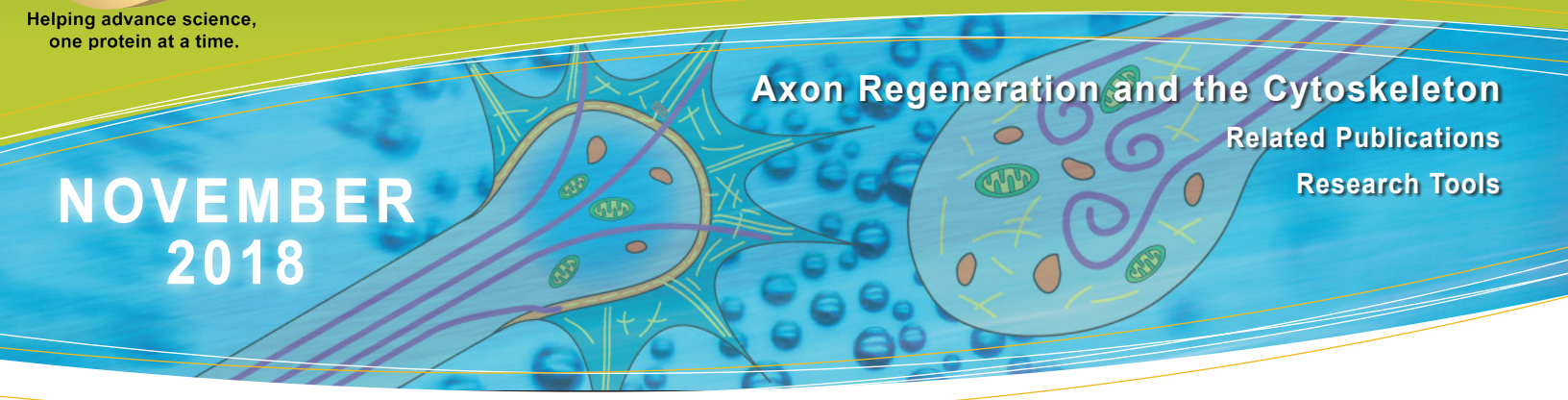

Axon Regeneration and the Cytoskeleton

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Axon Regeneration and the Cytoskeleton

The cytoskeleton of adult neurons in the central nervous system (CNS) is composed of different structural proteins, including microtubules (MTs) and F-actin. Normal cellular functions (e.g., morphology, motility, development, transport) rely upon the dynamicity of MTs and F-actin. Additionally, cytoskeletal dysfunction underlies many CNS diseases and injuries. For instance, the cytoskeletal dynamics in the adult CNS following axonal injury are not conducive to growth cone formation, axon regeneration, and recovery of function. To understand the role of the MT and F-actin cytoskeleton in adult CNS injury recovery, and how to modulate them for axon regeneration demands an understanding of cytoskeletal dynamics following axonal injury[1,2]. This newsletter discusses the roles of the MT and F-actin cytoskeleton in axon regeneration in the adult CNS.

The dogma is that axon regeneration does not occur in adult CNS neurons under physiological conditions. In contrast, damaged axons in the peripheral nervous system (PNS) are regenerated following injury. The focus on understanding this difference centers on two prominent regeneration-associated signaling cascades in the adult CNS: 1. activation of intrinsic regeneration-associated genes [RAGs]), and 2. external cues that inhibit axon re-growth[1,2].

Axon regeneration requires adult neurons to form new growth cones at the tips of damaged axons to initiate re-growth. This plasticity requires dynamic re-organization of the MT and F-actin cytoskeleton (Fig. 1). Axon regeneration in the PNS serves as a useful guide for how MTs and F-actin contribute to intracellular and extracellular signaling involved in axon regeneration in response to injury[1,2]. Importantly, there are several cellular responses to axotomy in PNS that either do not occur or are severely diminished in CNS once neurons reach adulthood. Axonal injury triggers an influx of calcium which activates a cascade of calcium-mediated signaling pathways[3-8], including activation of HDAC5-mediated deacetylation of axonal MTs, a post-translational modification necessary for axon regeneration[9-11]. Another intracellular injury-induced change that relies upon MTs is retrograde transport of proteins locally translated at or near the site of injury (e.g., assorted kinases and transcription factors) to the cell body for nuclear localization and presumable activation of RAGs[1,2]. Protein synthesis and MT-mediated anterograde transport of vesicles, organelles (e.g., mitochondria), and RAGs (e.g., cytoskeletal proteins, synaptic proteins) also occurs[1,2,10,12-14].

The injury-induced changes described above are necessary for growth cone formation and regeneration of injured axons (Fig. 1). Damaged axons of the PNS develop a new growth cone, which consists of stable MTs in the central (C-) domain and dynamic MTs in the tip of the cone, the peripheral (P-) domain[1,2,15]. As opposed to regenerative processes in the PNS, distal tips of injured CNS axons are capped by a dystrophic structure termed the retraction bulb which prevents axon regeneration. The retraction bulb

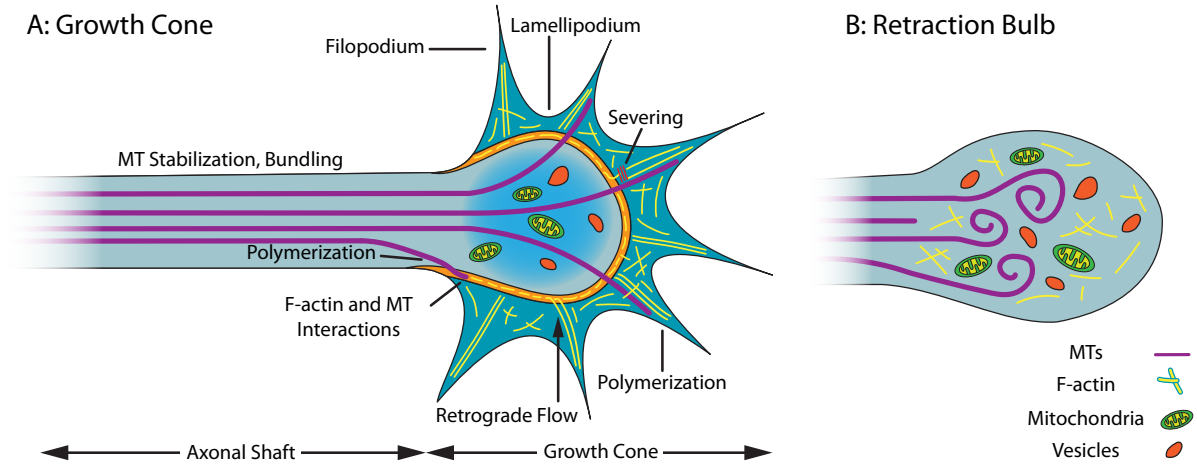

Figure 1. MT and F-actin cytoskeleton organization in growth cones and retraction bulbs in neurons. In regenerating axons (left panel), stable MTs are in the shaft (C-domain), while the growth cones contain dynamic MTs and F-actin (P-domain). The dynamicity of MTs and F-actin enables the formation and extension/withdrawal of lamellipodia and filopodia which are necessary for axon elongation. Retraction bulb (right panel) formation at the tip of the damaged axon prevents axon regeneration. In the bulb, the separate domains are lost, and the MTs are either depolymerized or disorganized.

TUBULIN AND ACTIN PRODUCTS

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consists of depolymerized MTs, disorganized MTs, no separate domains of actin and tubulin polymers, and no organized distribution of stable and dynamic polymers[1,2,15] (Fig. 1). Destabilization of a growth cone's organized MT network converts it into a retraction bulb-like structure. These findings suggest that dysfunctional MT dynamics contribute to the inability of adult CNS neurons to regenerate injured axons under physiological conditions[1,2,15,16]. As might be predicted, stabilization of MTs in the retraction bulb results in increased MT polymerization in the adult CNS axon tip, concomitant with decreased retraction bulb formation and initiation of growth cone development[1,2,16-19]. Dysregulated MT dynamics are also involved in the engagement of growth-inhibitory signaling cascades within astrocytes and fibroblasts that activate these fibrotic scar-forming cells at the damaged axon[1,16]. Upon taxol stabilization of MTs, release of extracellular matrix proteins from fibroblasts and growth-inhibitory proteins from astrocytes is prevented[1,16,17]. Thus, MTs are involved in pro- and anti-regenerative functions.

Actin also has a role in adult CNS responses to axonal injury, but it is less defined than that of MTs. Inhibition of the actin binding protein, non-muscle myosin II, results in significant reorganization of the actin cytoskeleton, predictably decreasing F-actin levels, increasing filopodia formation, and enhancing MT protrusions into the P-domain. These and other effects ultimately result in axon regeneration[1,20]. At least one pathway by which actin affects axon regeneration likely involves the RhoA GTPase, possibly by linking extracellular inhibitory signals to re-organization of the actin cytoskeleton[1,21-23].

Summary

Fully understanding how to regulate dynamically the MT and F-actin cytoskeleton in axon regeneration is essential in solving the riddle of re-growing damaged axons in adult CNS. Based on current research, the roles of the MTs and F-actin cytoskeleton in adult CNS axon regeneration will be at least three-fold: 1. Enhance the intrinsic regenerative capabilities of damaged neurons, 2. Provide tracks for anterograde and retrograde transport of injury-associated molecules and organelles, and 3. Modulate the release of molecules involved in fibrosis[1]. Selectively activating and inhibiting the MT and F-actin cytoskeleton offers great potential as treatments for injured axons in adult CNS neurons. To assist scientists in these pursuits, Cytoskeleton, Inc., offers a wide array of research tools, including purified actins, tubulins, and small GTPases, as well as functional assay kits for these proteins and live cell imaging reagents for MTs and F-actin.

Actin Biochem Kits

Product	Reactions	Cat. #
Actin Polymerization Biochem Kit (fluorescence format): rabbit skeletal muscle actin	30-100	BK003
Actin Binding Protein Spin-Down Assay Biochem Kit Rabbit skeletal muscle actin	30-100	BK001
Actin Binding Protein Spin-Down Assay Biochem Kit human platelet actin	30-100	BK013
G-Actin/F-actin In Vivo Assay Biochem Kit	30-100	BK037

Tubulin Biochem Kits

Product	Reactions	Cat. #
Tubulin Polymerization Assay Biochem Kit (absorbance format), porcine tubulin	24-30	BK006P
Tubulin Polymerization Assay Biochem Kit (fluorescence format): 99% pure porcine tubulin	96	BK011P
Microtubule Binding Protein Spin-Down Assay Biochem Kit	50-100	BK029
Microtubule/Tubulin In Vivo Assay Biochem Kit	30-100	BK038

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G-LISA Activation Assay Kits

Product	Reactions	Cat. #
G-LISA RhoA Activation Assay Biochem Kit (colorimetric format)	96	BK124
G-LISA RhoA Activation Assay Biochem Kit (luminescence format)	96	BK121

Tubulin and Actin Live Cell Reagents

Description	Ex / Em	Amount	Cat. #
SiR700-Actin Kit Includes Verapamil	690 / 720 nm	35 nmol	CY-SC013
SiR-Actin Kit Includes Verapamil	630 / 680 nm	50 nmol	CY-SC001
SiR700-Tubulin Kit Includes Verapamil	690 / 720 nm	35 nmol	CY-SC014
SiR-Tubulin Kit Includes Verapamil	630 / 680 nm	50 nmol	CY-SC002
Cytoskeleton Kit Includes SiR-Actin, SiR-Tubulin, and Verapamil	630 / 680 nm	50 nmol each	CY-SC006