

REVIEW

Neuronal cytoskeleton in synaptic plasticity and regeneration

Phillip R. Gordon-Weeks* and Alyson E. Fournier†

*The MRC Centre for Developmental Neurobiology, New Hunt's House, Guy's Campus, King's College London, London, UK

†Department of Neurology and Neurosurgery, Montreal Neurological Institute, 3801 Rue University, Montréal, QC, Canada

Abstract

During development, dynamic changes in the axonal growth cone and dendrite are necessary for exploratory movements underlying initial axo-dendritic contact and ultimately the formation of a functional synapse. In the adult central nervous system, an impressive degree of plasticity is retained through morphological and molecular rearrangements in the pre- and post-synaptic compartments that underlie the strengthening or weakening of synaptic pathways. Plasticity is regulated by the interplay of permissive and inhibitory extracellular cues, which signal through receptors at the synapse to regulate the closure of critical periods of developmental plasticity as well as by acute changes in plasticity in response to experience and

activity in the adult. The molecular underpinnings of synaptic plasticity are actively studied and it is clear that the cytoskeleton is a key substrate for many cues that affect plasticity. Many of the cues that restrict synaptic plasticity exhibit residual activity in the injured adult CNS and restrict regenerative growth by targeting the cytoskeleton. Here, we review some of the latest insights into how cytoskeletal remodeling affects neuronal plasticity and discuss how the cytoskeleton is being targeted in an effort to promote plasticity and repair following traumatic injury in the central nervous system.

Keywords: actin, axon regeneration, cytoskeleton, microtubule, neuronal plasticity.

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During CNS development, growth cones innervate their targets and neuronal connectivity is established with post-synaptic cells through formation of synapses. The growth cones of extending axons are highly dynamic and plastic integrating information from multiple attractive and repellent cues along their path to an appropriate target (Kolodkin and Tessier-Lavigne 2011). Ultimately, intracellular signals that are initiated in response to attractive and repellent cues converge on the cytoskeleton to regulate the direction and speed of outgrowth (Vitriol and Zheng 2012). The formation of a stable axo-dendritic synapse is then initiated through physical contact between an exploratory axonal growth cone and an elaborating dendrite from the post-synaptic cell, which ultimately forms a dendritic spine as a synaptic substrate. The exploratory behavior of the dendrite during synaptogenesis is also highly reliant on cytoskeletal dynamics (Tolias *et al.* 2011). Following establishment of a synapse, experience and activity during development and extending into adulthood regulate changes in spine shape that are driven by cytoskeletal reorganization. Thin-, stubby-, mushroom-, or branched-shaped spines are actively studied

as correlates of synaptic strength and maturity. Microtubules and actin filaments are present in dendrites and dendritic spines and are both dynamically regulated to regulate dendritic morphology and the complement of cell surface molecules at the synapse (Stepanova *et al.* 2003; Korobova and Svitkina 2010). Intriguingly, repellent cues that have been actively studied for their roles in suppressing axon regeneration following CNS injury have emerged as repressors of synaptogenesis and synaptic plasticity (Lee

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Address correspondence and reprint requests to Professor Phillip R. Gordon-Weeks, The MRC Centre for Developmental Neurobiology, New Hunt's House, Guy's Campus, King's College London, London, SE1 1UL, UK. E-mail: phillip.gordon-weeks@kcl.ac.uk (or) Dr. Alyson Fournier, Montreal Neurological Institute, BT-109, 3801 Rue University, Montréal, QC, H3A 2B4, Canada. E-mail: alyson.fournier@mcgill.ca

Abbreviation used: CRMP, Collapsin Response Mediator Protein; CSPGs, chondroitin sulfate proteoglycans; LTD, long-term depression; LTP, long-term potentiation; MAIs, Myelin-Associated Inhibitors; MAPs, Microtubule associate proteins; PSD, post-synaptic density.

et al. 2008; Raiker *et al.* 2010; Wills *et al.* 2012; Akbik *et al.* 2013). The role for these proteins in suppressing axon regeneration is mediated through the suppression of cytoskeletal rearrangements required for axon growth and it is reasonable to speculate that some conserved mechanisms might regulate synaptic rearrangements (Nash *et al.* 2009). Here, we review recent studies describing the role of the cytoskeleton in regulating synaptic plasticity and in restricting axonal repair following CNS injury. We discuss current strategies aimed at targeting the cytoskeleton to promote axonal repair following CNS injury and discuss some of the open questions in the field.

Actin and actin-regulatory proteins in synaptic plasticity

The post-synaptic elements of the majority of excitatory (glutamatergic) synapses on cortical and hippocampal neurons are formed by small, actin-rich protrusions called dendritic spines that emerge from the shafts of dendrites. Neuronal activity, particularly synaptic activity, regulates the density, size, and morphology of dendritic spines in developing and adult brain. The synapses on dendritic spines are capable of changing their long-term synaptic strength as a consequence of the pattern of their synaptic activity. Synapse strength can either be enhanced, by short bursts of high-frequency input, leading to long-term potentiation (LTP), or reduced, by prolonged low-frequency input, causing long-term depression (LTD). Changes in synaptic strength are associated with morphological changes at the synapse, particularly in the shape of the dendritic spine (Rochefort and Konnerth 2012). Increases in synaptic strength are associated with spine enlargement, whereas a reduction in synaptic strength correlates with smaller, simpler spines. Changes in dendritic spine morphology are driven by reorganization of the spine cytoskeleton: principally the actin filament (F-actin) and microtubule components. F-actin increases in response to local electrical stimulation or glutamate uncaging at individual spines (Okamoto *et al.* 2004; Honkura *et al.* 2008). LTD and LTP can bidirectionally modify the dynamics of the actin cytoskeleton in dendritic spines (Okamoto *et al.* 2004; Honkura *et al.* 2008). LTP promotes actin filament polymerization in spines leading to an increase in F-actin stability and a consequent enlargement of the spine, whereas LTD has the opposite effect, reducing F-actin levels and stability, leading to a reduction in spine volume. F-actin dynamics in dendritic spines are thought to be regulated by actin-binding proteins, including actin depolymerizing factor/cofilin, CaMKII (Ca²⁺/calmodulin-dependent protein kinase II), drebrin, and myosin II, and by diffusion of G-actin between spines and dendritic shafts (Lin and Webb 2009). F-actin in dendritic spines is directly regulated by activity in spines under the regulatory activity of calcium-dependent kinases and Rho GTPases (Saneyoshi and Hayashi 2012).

Microtubules and microtubule-associated proteins in synaptic plasticity

Dynamic microtubules in dendritic shafts can transiently enter dendritic spines and dendritic filopodia, the precursors of spines (Gu *et al.* 2008; Hu *et al.* 2008; Jaworski *et al.* 2009; Dent *et al.* 2011). The frequency of invasion is low, with approximately 1% of dendritic protrusions containing a microtubule at any one time (Hu *et al.* 2008) and the dwell time is in the order of minutes but, importantly, dwell time and the frequency of invasion are enhanced by synaptic activity (Gu *et al.* 2008; Hu *et al.* 2008; Mitsuyama *et al.* 2008; Jaworski *et al.* 2009). Microtubules invading spines are oriented with their plus-ends, the end at which tubulin assembly occurs preferentially, distal, i.e., directed toward the spine head. This behavior is reminiscent of microtubules invading the filopodia of growth cones (Geraldo and Gordon-Weeks 2009). In both cases, the microtubule-binding +TIP protein EB3 is located at the tip of the growing microtubule. Consequently, the +TIP protein EB3 can be used as a dynamic marker for microtubules invading dendritic spines. Microtubule invasion can occur from either the proximal or the distal dendrite shaft (Hu *et al.* 2008), consistent with the mixed orientation of microtubules in dendrites (Baas and Lin 2011). Microtubule invasion into spines correlates with an increase in F-actin and spine enlargement. Consistent with these findings, knockdown of EB3 reduces spine development (Gu *et al.* 2008; Jaworski *et al.* 2009). LTD, elicited by activation of NMDA receptors, inhibits microtubule entry into dendritic spines and leads to spine shrinkage (Kapitein *et al.* 2011). Importantly, these effects can be antagonized by over-expression of EB3 (Kapitein *et al.* 2011).

In addition to the role of the cytoskeleton in mediating structural rearrangements, the cytoskeleton may play a critical role in defining the molecular complement of the synapse. An early event in dendritic spine enlargement associated with increased synaptic strength is an enlargement of the post-synaptic density (PSD) and a concomitant increase in PSD-95, the major scaffolding protein of the PSD. Exposure of dendritic spines to brain-derived neurotrophic factor induces an increase in PSD-95 in the PSD along with spine maturation and this has been shown to depend on invasion of dendritic spines by microtubules (Hu *et al.* 2011). As the microtubule-binding +TIP protein EB3 can bind to PSD-95 directly through its src homology 3 (SH3) domain, microtubules invading dendritic spines might interact directly with the PSD (Sweet *et al.* 2011).

Open Questions in the field of the cytoskeleton in synaptic plasticity

Microtubule invasion of dendritic spines influences F-actin dynamics as indicated by the observation that the F-actin stabilizing compound jasplakinolide reverses EB3

knockdown in dendritic spines (Jaworski *et al.* 2009). However, the molecular mechanism linking dynamic microtubules to *F*-actin stability remains unknown. Microtubules and actin do not bind directly to each other, rather they interact through proteins that mediate interactions between the cytoskeletal elements (Geraldo and Gordon-Weeks 2009; Dent *et al.* 2011). A number of candidate proteins that might be capable of coordinating the actin and microtubule cytoskeleton are expressed in dendritic spines, including drebrin (Dun and Chilton 2010), IQ motif-containing GTPase-activating protein 1 (IQGAP1; [Jausoro *et al.* 2012]), and p140Cap (Jaworski *et al.* 2009), but their role has yet to be investigated (Georges *et al.* 2008). All three proteins can interact with microtubule-binding +TIP proteins: drebrin and p140Cap with EB3 (Geraldo *et al.* 2008; Geraldo and Gordon-Weeks 2009; Jaworski *et al.* 2009) and IQGAP1 with cytoplasmic linker protein 170 (CLIP-170) (Jausoro *et al.* 2013). In growth cones, drebrin links dynamic microtubules to actin filaments in filopodia by binding to EB3 (Geraldo *et al.* 2008; Worth *et al.* 2013). An interaction between drebrin and EB3 that links F-actin to microtubules has been independently confirmed in polarizing epithelial cells (Bazellieres *et al.* 2012), suggesting that the drebrin/EB3 pathway is canonical. Interestingly, microtubule invasion into dendritic spines is enhanced by over-expression of drebrin and, conversely, reduced by knockdown of drebrin (Merriam and Dent, personal communication). There is evidence that drebrin plays a role in synaptic plasticity (Ivanov *et al.* 2009a). For example, drebrin plays a role in activity-dependent redistribution of NMDA-type glutamate receptors at the synapse that is important for memory formation (reviewed in: Ivanov *et al.* 2009b). Over-expression of drebrin leads to the formation of long, thick dendritic spines (Mizui *et al.* 2005) and transgenic mice lacking the adult form of drebrin (drebrin A) show memory impairment (Aoki *et al.* 2009). The actin-binding protein IQGAP1 is involved in the regulation of the density and morphology of dendritic spines (Gao *et al.* 2011; Jausoro *et al.* 2013) and dendrite arborization, through an interaction with the +TIP protein CLIP-170 (Swiech *et al.* 2011). Furthermore, transgenic mice lacking IQGAP1 have a phenotype that includes memory defects and impaired LTP (Gao *et al.* 2011). However, more work is needed to define how IQGAP1 might regulate interactions between dynamic microtubules and the actin cytoskeleton in dendritic spine plasticity.

The Cytoskeleton and Regeneration

Damaged axons in the adult CNS fail to spontaneously regenerate following trauma or in the context of neurodegenerative disease in part because environmental cues actively inhibit axonal repair. Components of the glial scar such as chondroitin sulfate proteoglycans (CSPGs), repellent guidance cues, and myelin-associated inhibitors (MAIs)

including myelin-associated glycoprotein, oligodendrocyte-myelin glycoprotein, and Nogo all signal through receptors on injured axons to block regrowth (Silver and Miller 2004; Nash *et al.* 2009; Giger *et al.* 2010). Intriguingly, many proteins originally identified for their role in blocking nerve cell repair following trauma have been subsequently implicated in regulating synapse formation and synaptic plasticity and it is reasonable to speculate that some of these effects are through targeted regulation of the cytoskeleton. For example, Nogo-A and Oligodendrocyte-myelin glycoprotein suppress long-term potentiation (Raiker *et al.* 2010). These molecules bind to members of the Nogo receptor (NgR) family, which regulate dendritic spine shape and play a critical role in limiting the formation of new synapses in post-synaptic neurons (Lee *et al.* 2008; Wills *et al.* 2012). Furthermore, expression of NgR family members is down-regulated by neuronal activity and this may play a role in facilitating activity-dependent synapse development and in regulating anatomical plasticity in the adult (Wills *et al.* 2012; Akbik *et al.* 2013). Genetic deletion experiments have also demonstrated that the Nogo-NgR axis contributes to closure of the critical period of ocular dominance plasticity (McGee *et al.* 2005). An emerging strategy to overcome the influence of neurite outgrowth inhibitory molecules is to target intracellular substrates within injured axons. This approach is attractive because it may simultaneously overcome the influence of multiple inhibitory molecules in the CNS environment. Targeting individual inhibitory ligands or their axonally expressed receptors through genetic knockout studies has resulted in modest or negligible regeneration (Kim *et al.* 2003, 2004; Song *et al.* 2004; Ji *et al.* 2008; Nakamura *et al.* 2011).

The actin cytoskeleton and CNS repair

There is good evidence that inhibitory ligands converge upon the actin and microtubule neuronal cytoskeleton to block axonal repair. Acute responses to inhibitory cues are known to rely on the cytoskeleton because disruption of actin filament formation or microtubule dynamics attenuates repulsive turning responses to CSPGs (Challacombe *et al.* 1996, 1997). There is also clear evidence that inhibitory molecules actively initiate intracellular signals that initiate cytoskeletal rearrangements. Many inhibitory ligands activate the small GTPase RhoA and its downstream effector Rho Kinase (ROCK), master regulators of the actin cytoskeleton, to limit repair (Alabed *et al.* 2006; McKerracher and Higuchi 2006). Blockade of RhoA or ROCK promotes the growth of CNS neurons on repellent substrates both *in vitro* and *in vivo* (Wahl *et al.* 2000; Dergham *et al.* 2002; Borisoff *et al.* 2003; Fournier *et al.* 2003; Yukawa *et al.* 2005; Alabed *et al.* 2006). BA-210, a drug that blocks activation of RhoA, has been tested in a Phase I/IIa clinical study for spinal cord injury with some promising results

although the patient cohort was small (Fehlings *et al.* 2011). There is further evidence demonstrating that neuronal RhoA activation on myelin substrates regulates the phosphorylation and activity of the actin depolymerization factor, cofilin, and subsequent actin dynamics (Hsieh *et al.* 2006). Dorsal root ganglion neurons transduced with dominant negative LIM kinase (LIM domain kinase) exhibit attenuated responses to myelin inhibitors *in vitro* including diminished cofilin phosphorylation and neurite outgrowth inhibition. Non-muscle myosin II, an actin-binding protein that mediates retrograde actin flow and actin filament organization, is phosphorylated downstream of CSPGs and is also a critical regulator of neurite outgrowth inhibition (Yu *et al.* 2012). Treatment of cerebellar granule neurons or dorsal root ganglion neurons with the myosin II inhibitor blebbistatin or knockdown of myosin IIA or IIB attenuates inhibitory responses to CSPGs and myelin inhibitors (Hur *et al.* 2011b; Yu *et al.* 2012). While non-muscle myosin II is a substrate of ROCK, the effects of blebbistatin appear to be more robust than those of the ROCK inhibitor Y27632, suggesting that some effects are independent of the ROCK pathway. Interestingly, the growth promoting activity of blebbistatin requires dynamic rearrangements of both the actin and microtubule cytoskeletons (Hur *et al.* 2011b). An intriguing area of future investigation will be how the complex interplay between actin and microtubules regulates neuronal responses to inhibitory cues. For example, drugs that loosen the actin arc structure, such as ROCK inhibitors, promote advance of the microtubule-rich central domain (Zhang *et al.* 2003).

The microtubule cytoskeleton and CNS repair

There is compelling data demonstrating that drugs that stabilize the microtubule cytoskeleton promote CNS regeneration (Hellal *et al.* 2011; Sengottuvel *et al.* 2011). Retraction bulbs that form at the tips of injured CNS neurons are characterized by a disorganized microtubule cytoskeleton in comparison to the appropriately bundled microtubules evident in regenerating neurons of the PNS (Erturk *et al.* 2007). These observations evoked the idea that microtubules themselves may represent therapeutic targets for regeneration. Consistent with this prediction, Bradke and colleagues demonstrated that intrathecal taxol treatment promotes growth of serotonergic neurons of the raphe-spinal tract following dorsal hemisection or contusion injuries (Hellal *et al.* 2011). Similarly, taxol promotes the regeneration of injured retinal ganglion cells (Sengottuvel *et al.* 2011). In both cases, it is likely that taxol affects many aspects of the CNS response to injury including, but not limited to, suppressing formation of the glial scar, enhancing axon extension, and reducing inflammation. The necessity for neuronal $\text{mec-7}/\beta$ -tubulin for regeneration of mechanosensory neurons in *Caenorhabditis elegans* (*C. elegans*) suggests that taxol effects on neuronal tubulin likely contribute to the

repair (Kirszenblat *et al.* 2013). These results are intriguing and ultimately the identity of substrates that can remodel the microtubule cytoskeleton may lead to therapeutic strategies with greater specificity and limited off-target effects.

Dynamic properties of tubulin are controlled in part by post-translational modifications. Acetylated tubulin is enriched in stable microtubules, whereas tyrosinated tubulin is enriched in dynamic microtubules. In the peripheral nervous system, where neurons do regenerate, it has been shown that there is a reduction in acetylated tubulin in the proximity of the injury site, which is mediated by histone deacetylase 5 and which is absent in injured CNS neurons (Cho and Cavalli 2012). When sciatic nerve ligations were performed in the presence of the HDAC inhibitor scriptaid, regeneration was markedly inhibited (Cho and Cavalli 2012). Pan-HDAC inhibitors and HDAC-6-selective inhibitors or HDAC-6 siRNA attenuate neuronal responses to Myelin-associated glycoprotein and CSPGs *in vitro* (Rivieccio *et al.* 2009). HDAC-6 targets relevant to this effect have not been identified, but the effects are independent of transcription raising the possibility that some of these effects could be attributed to tubulin deacetylation.

Microtubule associate proteins (MAPs) also play a crucial role in regulating microtubule dynamics. Microtubule plus end-tracking proteins (+TIPs) accumulate at the tips of microtubules and several including adenomatous polyposis coli and cytoplasmic linker proteins have been shown to promote axonal formation and/or elongation of embryonic neurons through microtubule stabilization (Zhou *et al.* 2004; Neukirchen and Bradke 2011). In the case of CLASP, it has been shown that transfection of shRNA targeting CLASP1 and CLASP2 limit peripheral regeneration of adult Dorsal root ganglia (DRG) neurons following sciatic nerve crush (Hur *et al.* 2011a). The role of these proteins in regulating regenerative responses in the CNS remains to be addressed. MAPs of the collapsin response mediator protein (CRMP 1-5) family have also been identified as interesting targets for regulating CNS regeneration. The CRMPs are a family of cytosolic phospho-proteins that play important roles in neuronal differentiation and axonal growth and guidance. *C. elegans* CRMP (UNC-33) affects axon guidance, axon extension, and axon/dendrite specification (Yamashita and Goshima 2012). CRMP2 is phosphorylated in response to MAIs and over-expression of dominant negative CRMP2 attenuates myelin inhibition *in vitro* (Mimura *et al.* 2006). CRMP4 forms a complex with RhoA that is critical for transducing inhibitory information in response to inhibitory cues present in myelin and within the glial scar and CRMP4 knockdown promotes neurite growth on inhibitory substrates *in vitro* (Alabed *et al.* 2007; Nagai *et al.* 2012). Blocking the interaction between CRMP4 and RhoA with a molecular antagonist or through GSK3 β -dependent phosphorylation of CRMP4 also overcomes the inhibitory influence of MAIs (Alabed *et al.* 2007, 2010); however, the *in vivo* contribution

of CRMPs to neuronal repair has yet to be assessed. In addition to affecting MAPs that may directly affect microtubule dynamics, inhibition of the motor protein kinesin-5 with monastrol promotes the growth of DRG neurons into CSPG borders *in vitro* raising the possibility that targeting more efficient microtubule transport along the axon shaft may also be targeted to promote regeneration (Lin *et al.* 2011).

Open Questions in the field of cytoskeletal dynamics and CNS repair

Progress has also been made in the past few years in targeting regulatory processes that drive intrinsic growth programs in neurons. Combined genetic deletion of the signaling molecules SOCS3 (Suppressor of cytokine signaling 3) and PTEN (Phosphatase and tensin homolog) results in the most significant nerve regeneration ever observed following experimental spinal cord injury (Sun *et al.* 2011). The downstream effectors of these deletions have not been fully elucidated and there is good reason to believe that cytoskeletal rearrangements may contribute to these effects. Direct targeting of PTEN or SOCS3 may result in significant side effects; thus, it is critical to understand the molecular targets that drive regenerative growth and any contributions of the cytoskeleton. The impact of cytoskeletal manipulations on appropriate nerve cell targeting is also an open question that will need to be addressed as our ability to entice regeneration improves. Further studies in model systems such as *C. elegans* may further illuminate how cytoskeletal rearrangements may regulate regenerative growth. Using laser microsurgery to sever axons, several groups are now harnessing the power of genetics and live imaging to study fundamental mechanisms contributing to axon regeneration. In such a study, the depolymerizing kinesin-13 family member KLP-7 and the cytosolic carboxypeptidase-6 have been shown to play an important role in regulating microtubule rearrangements following injury (Ghosh-Roy *et al.* 2012). How these studies may translate the adult mammalian CNS, which is rich with neurite outgrowth inhibitors, remains an open question.

Concluding remarks

Cytoskeletal rearrangements are critical to regulate synaptic plasticity as well as plasticity and repair following CNS injury. It is apparent that understanding the cues and molecular mechanisms that regulate the two processes can coinform the two fields and lead to exciting insights into strategies to regulate plasticity in both contexts. Further defining the molecular mechanisms that coordinate and regulate the actin and microtubule cytoskeleton, while limiting side effects of interventions, are important future directions of research for the plasticity and repair fields.

Conflicts of interest

The authors have no conflict of interest to declare.

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