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INVITED REVIEW

Intra-axonal protein synthesis – a new target for neural repair?

Jeffery L. Twiss[1,*], Ashley L. Kalinski[1,†], Rahul Sachdeva[2,‡], John D. Houle[2]

1 Department of Biological Sciences, University of South Carolina, Columbia, SC, USA
2 Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA, USA

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Abstract

Although initially argued to be a feature of immature neurons with incomplete polarization, there is clear evidence that neurons in the peripheral nervous system retain the capacity for intra-axonal protein synthesis well into adulthood. This localized protein synthesis has been shown to contribute to injury signaling and axon regeneration in peripheral nerves. Recent works point to potential for protein synthesis in axons of the vertebrate central nervous system. mRNAs and protein synthesis machinery have now been documented in lamprey, mouse, and rat spinal cord axons. Intra-axonal protein synthesis appears to be activated in adult vertebrate spinal cord axons when they are regeneration-competent. Rat spinal cord axons regenerating into a peripheral nerve graft contain mRNAs and markers of activated translational machinery. Indeed, levels of some growth-associated mRNAs in these spinal cord axons are comparable to the regenerating sciatic nerve. Markers of active translation tend to decrease when these axons stop growing, but can be reactivated by a second axotomy. These emerging observations raise the possibility that mRNA transport into and translation within axons could be targeted to facilitate regeneration in both the peripheral and central nervous systems.

Key Words: *mRNA transport; translational control; RNA binding protein; axon regeneration; spinal cord injury; peripheral nerve injury*

***Correspondence to:**
Jeffery L. Twiss, M.D., Ph.D., twiss@mailbox.sc.edu.

† Current address for Ashley L. Kalinski is Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA.
‡ Current address for Rahul Sachdeva is International Collaboration on Repair Discoveries (ICORD), University of British Columbia, Vancouver, British Columbia, Canada.

orcid:
0000-0001-7875-6682 (Jeffery L. Twis)

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Post-transcriptional regulation provides a means to fine tune gene expression. Since a single messenger RNA (mRNA) can be translated into several copies of a protein, controlling the rate of translation for individual mRNAs can have major effects on the levels of a protein generated. The stability of an individual mRNA also directly impacts the amount of that mRNA available for translation. Proteins and small non-coding mRNAs (*e.g.*, RNA binding proteins [RBP] and micro-RNAs [miRNA], respectively) interact with mRNAs and modulate their translation and stability. RBPs also play a role in subcellular localization of mRNAs. Polarized cells use mRNA localization to introduce new proteins in distinct subcellular domains. Neurons are highly polarized and they use mRNA transport and localized translational control in both their dendrites and axons. Protein synthesis in dendrites has largely been associated with synaptic efficacy. With the much greater distance that axons traverse, localized protein synthesis would be an appealing mechanism for the distal axon to locally regulate its protein levels. However, initial ultrastructural studies showing polyribosomes in dendrites failed to detect these in the axonal compartment, suggesting that ribosomes and other translational machinery are excluded from axons. With substantial advances in molecular tools and cellular techniques over the last two decades, many groups have now unequivocally shown that axons have the capacity to synthesize proteins (see Perry and Fainzilber, 2014 and references within).

In the peripheral nervous system (PNS), intra-axonal protein contributes to axon regeneration. Studies from the Fainzilber group indicate that synthesis of Importin β1, RanBP1, vimentin, and Stat3α proteins in distal axons is used to signal injury to the cell body (Perry and Fainzilber, 2014). Importin β1 (KPNB1) is a member of the karyopherin protein family that imports proteins across the nuclear

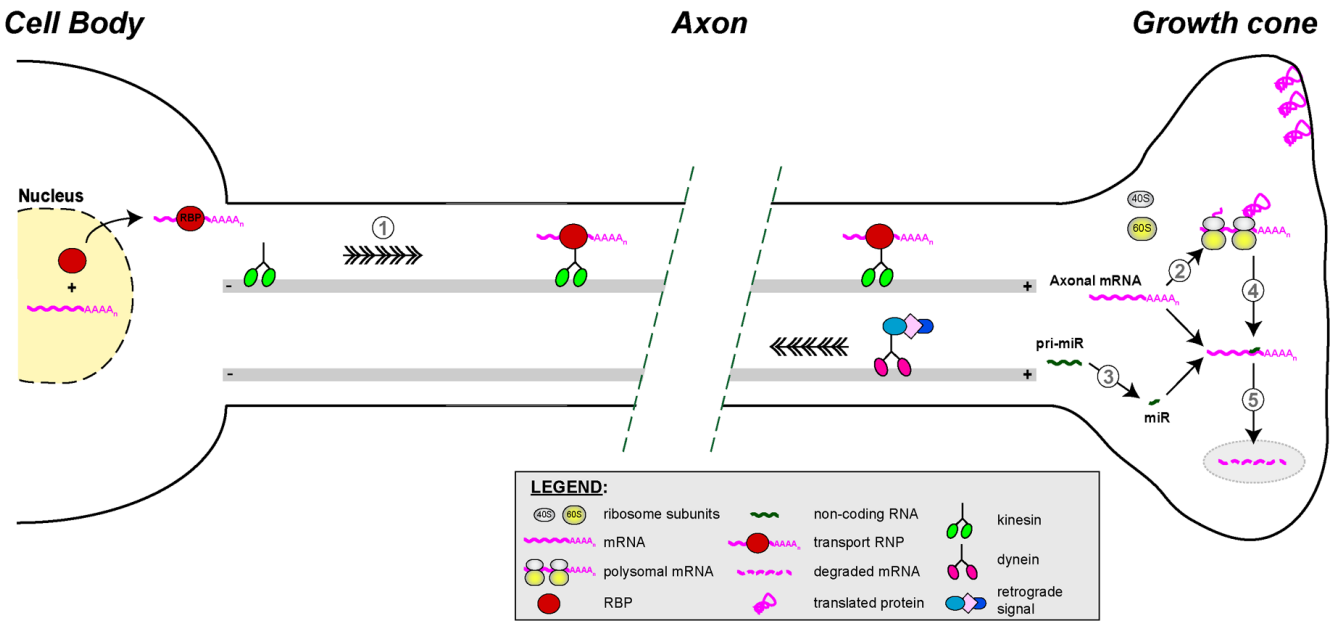

Figure 1 Molecular targets for modulating intra-axonal protein synthesis.
This schematic outlines mechanisms that could be targeted for exogenously regulating axonal protein synthesis to potentially increase axon regeneration. These targets include: (1) mRNA transport; (2) translational control; and (3) miRNA maturation with subsequent repression of mRNA translation (4) or degradation of bound mRNAs (5).

membrane. The axonally-generated Importin β1 protein dimerizes with cell body-synthesized Importin α3 protein that arrives in axons through anterograde transport. Together with dynein motor protein, the Importin β1/α3 heterodimer transports signaling proteins to the nucleus to modulate an injury-induced transcriptional response (Perry et al., 2012). Dimerization of these two proteins is made possible by intra-axonal translation of Importin β1 that is activated by increases in axoplasmic Ca^{2+} after injury. This increase in axoplasmic Ca^{2+} also triggers translation of RanBP1 mRNA in axons, whose protein product regulates a RanGTPase that frees axonal Importin α3 for binding to the newly translated Importin β1 protein (Yudin and Fainzilber, 2009). On the other hand, axonally synthesized GAP43 and β-actin proteins are used locally for growth of sensory axons, and changes in the amount of GAP43 or β-actin mRNA targeted into axons contributes to the type of axon outgrowth (see (Gomes et al., 2014 and references within). Other axonally synthesized proteins contribute to axon growth *in vitro*, but it is not clear whether they play the same role *in vivo* or not. For example, intra-axonal translation of RhoA mRNA has been linked to chondroitin sulfate proteoglycan (CSPG)-mediated growth inhibition in cultured neurons (Walker et al., 2012a). Beyond growth, locally generated proteins have been implicated in maintenance of axons, cell survival, and mitochondrial respiration in cultured neurons and sometimes *in vivo* for developing neurons (see Gomes et al., 2014; Perry and Fainzilber, 2014 and references within).

The functions outlined above for proteins synthesized in cultured neurons and *in vivo* in the developing nervous system and adult PNS could conceivably be harnessed for neural repair strategies once more is known of the molecular mechanisms regulating mRNA transport and translation. Recent works from several groups point to the possibility that adult CNS neurons also have the capacity for synthesizing proteins in their axons. Akins et al. (2012) have shown that axons in adult mouse have granular profiles of the fragile X mental retardation (FMRP) and fragile X related (FXR1, FXR2) RBPs. These tend to concentrate in regions of the brain with relatively higher plasticity like the olfactory nerve/bulb (Akins et al., 2012). Walker et al. (2012b) reported intra-axonal translation in adult mouse spinal cord by delivering an exogenous mRNA directly into axons using SinB is viral particles. In cultured neurons, delivering adenylate cyclase mRNA into axons with this method allowed for axon growth in the face of growth-inhibitory CSPGs. Using a peripheral nerve graft (PNG) into the adult rat spinal cord, Kalinski et al. (2015) showed that ascending spinal cord axons contain mRNAs and translational machinery as they are regenerating in the PNG. Levels of the translational machinery seem to decline as the axons reached ends of the PNGs and stop growing, suggesting that the axon's translational activity may reflect the growth supporting environment of the PNG (Sachdeva et al., 2016). Keeping with this notion, Selzer et al. (2016) recently showed that regenerating reticulospinal axons in the *Lamprey* spinal cord contain mRNAs and translational machinery. In contrast to higher vertebrates, some reticulospinal axons in the *Lamprey* can spontaneously regenerate after spinal cord injury. Together these studies indicate that, at least under some conditions, CNS axons have the mRNA transcripts and necessary translational machinery to generate new proteins.

It is tempting to speculate that mRNA translation in the spinal cord axons noted above is a reflection of regenerative growth. As noted the *Lamprey* reticulospinal axons can spontaneously regenerate, and the PNGs analyzed above similarly support regeneration compared to the non-permissive environment of the mammalian spinal cord (Kalinski et al., 2015; Selzer et al., 2016). So could the failure of early ultrastructural studies to visualize ribosomes in axons have resulted from investigators looking in the wrong place or under the wrong conditions? Recent work from the Hengst group is in support of this notion. Baleriola et al. (2014) showed that ATF4 mRNA is transported into adult hippocampal axons *in vivo*, where it is locally translated after application of exogenous amyloid-β peptide (Baleriola et al., 2014). While a provocative finding for the neurodegeneration field, this study emphasizes that these mammalian CNS neurons retain the capacity for intra-axonal protein synthesis into adulthood. Depending on the specific mRNA targets, activating mRNA transport into and translation within axons seems to be utilized for a neuron's injury responses or increasing its axon's intrinsic regeneration capacity.

We posit that the proteins and non-coding RNAs responsible for regulating the transport and translation of mRNAs are rising as prime candidates for neural repair strategies. For example, the levels of zip code binding protein 1 (ZBP1) in adult sensory neurons limits how much β-actin and GAP43 mRNAs can localize into axons (see Gomes et al., 2014 and references within). So increasing ZBP1 expression could effectively increase transport of the mRNAs encoding growth-promoting proteins. However, ZBP1 binds to many different mRNAs and there is a pressing need to determine which mRNAs it transports into axons and if CNS and PNS neurons differ in their use or need for ZBP1. Likewise, many more RBPs are undoubtedly used for transport and translation of axonal mRNAs; there is a similar need to identify the axonal RBPs and the cohorts of mRNAs they interact with. miRNAs have also been detected in axons in culture and PNS axons *in vivo*. These non-coding RNAs provide a platform for modifying the translation and stability of mRNAs within the axons. Interestingly, the Yoo group recently showed that miRNA precursors (pre-miRNAs) localize to axons of the sciatic nerve (Kim et al., 2015). This raises the possibility for another step of regulatory control for intra-axonal protein synthesis that could be a target for future neural repair strategies. However, more work is needed to define the mRNA targets for the axonal miRNAs as well as the signaling mechanisms that control pre-miRNA-to-miRNA maturation in axons.

In summary, localized protein synthesis clearly can occur in both PNS and CNS axons. Although there are increasingly new functions identified for axonally synthesized proteins, growth of the axon remains a key mechanism for the protein products of axonal mRNAs. The molecular events briefly outlined above that contribute to regulation of axonal mRNA transport and translation (**Figure 1**) could indeed bring new strategies to facilitate axon regeneration. Evidence has been mounting for this possibility in the PNS, and studies over the past two years suggest that protein synthesis can be activated in at least some axons in the brain and spinal cord. While RBPs and miRNAs offer potential targets for facilitating axon regeneration, more work is needed to understand the molecular mechanisms underlying their mRNA interactions and activities as well as the breadth of axonal mRNAs that they affect.

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