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To the End of the Line: Axonal mRNA transport and local translation in health and neurodegenerative disease

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Abstract

Axons and growth cones, by their very nature far removed from the cell body, encounter unique environments and require distinct populations of proteins. It seems only natural, then, that they have developed mechanisms to locally synthesize a host of proteins required to perform their specialized functions. Acceptance of this ability has taken decades; however, there is now consensus that axons do indeed have the capacity for local translation, and that this capacity is even retained into adulthood. Accumulating evidence supports the role of locally synthesized proteins in the proper development, maintenance, and function of neurons, and newly emerging studies also suggest that disruption in this process has implications in a number of neurodevelopmental and neurodegenerative diseases. Here, we briefly review the long history of axonal mRNA localization and local translation, and the role that these locally synthesized proteins play in normal neuronal function. Additionally, we highlight the emerging evidence that dysregulation in these processes contributes to a wide range of pathophysiology, including neuropsychiatric disorders, Alzheimer's, and motor neuron diseases such as Spinal Muscular Atrophy and Amyotrophic Lateral Sclerosis.

Keywords

Axonal mRNA; Local translation; RNA trafficking; Transport; Neurodegenerative disease

Introduction

The intra-axonal transportation of mRNA and concomitant local translation of protein has had a sciatic-length road to acceptance. Early data suggested there was no place for local translation in the axon, lacking both message and the means of production. Palay and Palade demonstrated in 1955 that there was a lack of ribonucleoprotein in histochemical preparations of neurons, suggesting that the requisite equipment for protein production was absent(Palay & Palade, 1955). A similar conclusion was reached by Deitch, Murray, and others suggesting that there was no organization of protein producing superstructures in axons (Deitch & Moses, 1957, Deitch & Murray, 1956). Therefore, why would there be mechanisms to transport mRNA to the axon if there was no ability to use these messages as

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templates for protein production? Changing these opinions would require robust and highly sensitive methodologies, many of which were inconceivable until the advent of modern techniques.

As early as the mid-1960s, Koenig and others suggested the presence of translational machinery and production of locally necessary proteins (e.g., acetylcholinesterase) in the axon. However, the dogma that mRNA's role was fulfilled only in the soma persisted, with heated debates playing out in the discussions of numerous papers (Capano, Giuditta et al., 1987, Jarlstedt & Karlsson, 1973, Zheng, Kelly et al., 2001). Many of these arguments centered on the use of model systems and methodologies employed. The use of the squid giant axon and Aplysia provided a reasonably accessible source of axoplasm; however, they were decried for being non-representative of adult mammalian neurons since they possess both great regenerative properties and atypical morphologies.

More convincing methodologies and findings began to appear in high-impact journals through the 1990s. Mohr et al. showed transport of transcripts encoding the neurotransmitters oxytocin and vasopressin to the axons of embryonic rat hippocampal and pituitary neurons (Mohr, Fehr et al., 1991). Later, Conner et al. suggested that both Brain Derived Neurotrophic Factor (BDNF) mRNA and protein are transported anterogradely down the axon, based on histological staining of hemispheric sections (Conner, Lauterborn et al., 1997). Moreover, subsequent studies also demonstrated a variety of other transcripts in the axons. The expression of these transcripts was miniscule in comparison to the gross production of proteins in the cell, but seemed to be important for controlling the expression of proteins under tight temporal and spatial controls.

While this evidence was promising, there were limitations to the conclusions that could be garnered from their results. Namely, the use of gross histological sections left ambiguity in the *in situ* hybridization patterns; were the mRNAs truly in the axons of neurons or present in glial cells? More conclusive evidence would come as the result of mammalian axoplasmic preparations and, critically, the ability to isolate living axons from their somas in cell culture (Eng, Lund et al., 1999, Hillefors, Gioio et al., 2007, Olink-Coux & Hollenbeck, 1996, Poon, Choi et al., 2006, Vogelaar, Gervasi et al., 2009, Wu, Hengst et al., 2005, Zheng et al., 2001). The seminal work detailing the use of poly-dimethyl siloxane (PDMS) polymer microfluidic devices to isolate axons provided the tool crucial to producing robust evidence of mRNA trafficking, and the related phenomenon of local translation (Taylor, Blurton-Jones et al., 2005).

A Highly Complex Population

The neuron's fundamental physiological role is to transmit information in a timely manner. Given neurons' dramatically polarized morphology, a variety of intricate chemical and electrical orchestrations are required to make this transmission possible. Perhaps then it is not surprising that the phenomena of mRNA transport and local translation are diverse both in the players involved and the mechanisms of control. The various nucleic acids, proteins, and control mechanisms involved serve to facilitate the timely transmission of vital information.

After the transcripts are made in the nucleus, there are a series of regulatory steps that control their journey to the axon terminal. Many of these transcripts are bound to RNA Binding Proteins (RBPs) along with various other transcripts. Axonally localized mRNA transcripts contain stereotyped motifs to which the RBPs bind. The archetypical RBP may be the Zipcode-Binding Protein 1 (ZBP-1) and its responsibilities in localizing β-actin mRNA to the distal axon. ZBP-1 binds to a 3′-UTR motif of β-actin mRNA, its "zipcode" encoding its axonal destination. The interaction between ZBP-1 and β-actin is critical for localization of the transcript and subsequent local translation (Donnelly, Willis et al., 2011, Sotelo-Silveira, Crispino et al., 2008, Welshhans & Bassell, 2011).

There are numerous other RBPs responsible for directing the localization of different transcripts, for example, the Splicing Factor, proline-glutamine rich (SPFQ) protein requires a short sequence that is Guanine and Uracil rich to localize transcripts to the axons of sensory neurons (Cosker, Fenstermacher et al., 2016). While many transcripts contain the requisite sequence, it is critical to the localization of the *laminb2* and *bclw* transcripts. Similarly, TDP-43 is another RBP that assists in both the processing and trafficking of transcripts (Alami, Smith et al., 2014). The requisite RBPs responsible for directing the localization of the majority of axonal transcripts have yet to be identified, and there is significant ongoing effort in this regard since RBPs are thought to be ideal targets for modifying axonal transport to enhance axon growth or as potential therapeutic targets for a number of neurological disorders.

Once mRNAs are stabilized by binding to RBPs in granules, they are prepared to make the long journey to the axon terminal. As with any journey, there is the quickest route and several other slower methods. The time-sensitive nature of axonal protein expression, especially in development, has produced sufficient selective pressure to favor the quickest route in most scenarios (Maday, Twelvetrees et al., 2014). RBPs have domains that associate with the microtubule-associated molecular motor Kinesin family of proteins. Different RBPs show varying levels of affinity for kinesin family members, suggesting another mechanism of control for axonally localized transcripts (Chevalier-Larsen & Holzbaur, 2006). Interestingly, these motors show both anterograde and retrograde motion when they are carrying their cargo, suggesting that they can modulate the levels of mRNA in specific neuronal subdomains on the fly (Brady, Pfister et al., 1990, Kanai, Dohmae et al., 2004, Waterman-Storer, Karki et al., 1997, Zhang, Pan et al., 2003). While specific bidirectional movement of RNA granules has been observed in both dendrites and in "neurites" in culture (Kanai et al., 2004, Knowles, Sabry et al., 1996) this has not been demonstrably shown in axons. Adding to the complexity, there is newly emerging evidence suggesting that local axonal translation of the motor components themselves is necessary for stimulus-induced retrograde transport of a number of cargos (Villarin, McCurdy et al., 2016). Again, RNA granules have not yet been shown to be carried by these locally synthesized motor components, but it is intriguing to speculate that axonal RNAs can act as both retrograde cargo and the template encoding the means of this transport.

The mRNA transcripts transported down the axon are a highly heterogeneous group, sensitive to both developmental timepoints and pathophysiological conditions. Previous work has shown that there are a number of transcripts that are localized to the axon and

exhibit altered expression levels in injury conditions. These include cytoskeletal structural elements (e.g. vimentin, peripherin), heat-shock proteins, Edoplasmic Reticulum proteins (e.g. Erp29, calreticulin), and disease-condition linked proteins (e.g. SP22, γ-synuclein) (Willis, Li et al., 2005). Profiling studies have also confirmed that axons contain transcripts encoding protein machinery, such as ribosomal proteins and translation regulatory factors (Gumy, Yeo et al., 2011, Taylor, Berchtold et al., 2009, Zivraj, Tung et al., 2010).

One of the most surprising findings has been the number of axonal mRNAs encoding membrane proteins and secreted proteins. Various profiling studies place the relative representation of transmembrane transcripts at between 7% and 24%, depending on the neuronal type, developmental stage, and injury conditioning (Gumy et al., 2011, Shigeoka, Jung et al., 2016, Sotelo-Silveira et al., 2008, Willis, van Niekerk et al., 2007, Zivraj et al., 2010). What makes these results so surprising is the apparent lack of secretory organelles in axons. Membrane proteins require processing through both the endoplasmic reticulum (ER) and the Golgi apparatus to facilitate proper protein folding and post-translational glycosylation. To date, neither rough ER (RER) nor Golgi apparatus have been convincingly detected in axons. It has been suggested that RER may be difficult to distinguish ultrastructurally in the large volume of the axon, particularly if these structures are sparsely present and intermingled with the dense cytoskeletal components of the axon. While there is little ultrastructural evidence for these structures, several studies have demonstrated the presence of components of the secretory organelles (Gonzalez, Canovas et al., 2016, Merianda, Lin et al., 2009, Vuppalanchi, Coleman et al., 2010). These components co-localize in a similar manner as they do in the canonical RER and Golgi structures, suggesting that at the very least there is a functional equivalent of these structures in axons. This is supported by the successful axonal translation and membrane insertion of functional G-protein coupled receptor in *Lymnaea* (Spencer, Syed et al., 2000), EphA2 receptors in severed chick axons (Brittis, Lu et al., 2002), and neuronal membrane protein 35 and κ-opioid receptor in mammalian peripheral nerve axons (Bi, Tsai et al., 2006, Merianda, Vuppalanchi et al., 2013). Thus, though the ultrastructural evidence of secretory organelles is still lacking, it appears that the abundant number of membrane mRNAs transported into the axons can be used as local templates for the synthesis of functional proteins.

The population of axonal mRNAs is dynamic. Work by Zivraj et al. and others (Gumy et al., 2011, Taylor et al., 2009, Zivraj et al., 2010) demonstrated that the axonal mRNA profile differs significantly in young and old neurons and that these changes are indicative of and essential for proper physiology. An exemplary case is the expression of the catenin receptor RNA in the axons of young neurons and its absence in older, established neurons. As a developmental signal, catenin is necessary for proper neuronal localization and turning, events that are completed once synapses have been formed. The ability to alter the locations of mRNAs and, by extension, the proteins they encode may influence their functionality. Providing another level of control, the mechanism of axonal transport is a convenient way for neurons to interact with their local environments under tight time constraints.

Developmental and post-developmental functions of axonally synthesized proteins

As alluded to, axonally synthesized proteins are important for the temporally-sensitive events in development, including axon growth and guidance, neuron specification and survival, and neuronal upkeep events like plasticity, injury response, axonal maintenance and retrograde signaling. Much as one would not construct a building then transport it, completed, cross-country so too does the neuron produce structures locally, rather than invest energy and time in transporting completed structures.

Developing neurons face a challenge unique to their cellular identity: successfully and rapidly arriving at their synaptic targets. Both Central Nervous System and Peripheral Nervous System neurons locally synthesize proteins in their axons as they weave their ways to their goals. The complexity of these local axonal events has recently been discussed in an excellent detailed review from the Hengst lab (Batista & Hengst, 2016). Here, we will highlight several cases that exemplify the breadth of these local events. One such locally synthesized protein is β-catenin, which not only shows the above-mentioned differential expression, but is also extremely important for both developmental gene expression and local changes to cytoskeletal elements at the growth cone. It has been shown to be important in Netrin-1 mediated growth and turning; with increased local translation of β-catenin in response to Netrin-1 exposure in the developing thalamus (Pratt, Davey et al., 2012).

Netrin-1 is an axonal guidance cue in developing mammalian nervous systems, impacting a variety of locally translated proteins during development. Another key target is β-actin, the local synthesis of which is integral to the ability of developing axons to turn in response to stimuli. It has been shown that there is an asymmetrical nature to the local synthesis of actin, functioning as a physiological analog to a set of reins, moving the axon closer to areas of attractive stimuli and away from repulsive or toxic stimuli. Actin's asymmetrical synthesis can be influenced by a number of other factors including BDNF (Welshhans & Bassell, 2011, Yao, Sasaki et al., 2006), Sema3A (Campbell & Holt, 2001, Campbell, Regan et al., 2001), and Sonic Hedgehog (Lepelletier, Langlois et al., 2017). In addition, targeting of β-actin mRNA has been shown to be directly influenced by signaling from these factors (Willis et al., 2007), suggesting that both the targeting of axonal mRNAs, and their subsequent translation, can be regulated by external cues.

Once the neuron has found its target, constant work is needed to mature and maintain the synapse both during the rest of development and throughout the life of the organism. Many of the important events of synaptic pruning and maturation are driven by elements that are locally synthesized in the distal axon. The role of local protein synthesis is underscored by a series of studies conducted in the early 2000s in which synapsing axons were severed from their somas. Interestingly, the synapse continued to function for nearly three days following the axonal isolation bolstered by the locally produced proteins (Meems, Munno et al., 2003, Spencer et al., 2000). Local translation is not only important for the maintenance of the synapse, but also for its formation. In a recent paper, the Hengst group has shown that assembly of the presynaptic terminal requires local protein synthesis, and that this local translation is temporally rapid, occurring within 15 minutes of signals that induce synapse

formation (Batista, Martinez et al., 2017). They further demonstrated that the transcript for the t-SNARE protein SNAP25 is recruited to sites of synapse induction, where it is subsequently locally translated. Blocking this local translation results in reduced synaptic vesicle release (Batista et al., 2017). Taken together, these early studies and more recent results highlight the importance of local translation in the development, maintenance, and function of presynaptic terminals.

Development is one of the two most significant constructive challenges placed on the architecture of the neuron; the other is injury. Shearing of the axon leads to different responses in the central and peripheral nervous systems. While most CNS axons will not regenerate, those in the PNS typically do. The regeneration seen in the PNS is, in part, facilitated by some of the same locally translated proteins that help a developing neuron find its synaptic endpoint and establish a functioning synapse (Cox, Hengst et al., 2008, Deglincerti & Jaffrey, 2012, Gumy et al., 2011, Taylor et al., 2009, Verma, Chierzi et al., 2005). Local translation following an injury is twofold, providing both the materials for axon regrowth as well as providing proteins that act as retrograde signals, reporting on the status of the injury and subsequent recovery (Ben-Yaakov, Dagan et al., 2012, Cox et al., 2008, Donnelly et al., 2011, Hanz, Perlson et al., 2003). Given its role in development, it is likely not surprising that a number of groups have shown the upregulation of actin production at the growth cone of regenerating axons (Michaelevski, Medzihradszky et al., 2010, Verma et al., 2005, Willis et al., 2007, Yudin, Hanz et al., 2008) as well as a number of other cytoskeletal elements like vimentin, actin-related protein, and neurofilament. Vimentin acts as both a cytoskeletal structural element and, interestingly, a retrograde signal to the soma of axonal injury. It helps transmit the injury signals begun by MAP kinases, specifically ERK1/2, in the axons back to the somas, by linking to these kinases (Perlson, Hanz et al., 2005).

Local protein translation can serve to amplify and accentuate nervous system-wide signals, and to facilitate communication between the axon and the neuronal cell body. For example, following axonal injury there is a complex and orchestrated response within the axon designed to generate an injury signal that allows the neuron to survive and ultimately regenerate. Local axonal translation is an integral and necessary component of this response. Axonal injury results in calcium influx, which initiates translation of mRNAs that are resident within the axon (Hanz et al., 2003, Rishal & Fainzilber, 2014). Among these is Importin β1, which is translated in response to injury and forms the key member of the retrograde signaling complex (Hanz et al., 2003). Blocking either the retrograde transport of the injury signaling complex or deleting the axonal localizing signal within the Importin β1 transcript delays axonal regeneration and functional recovery following injury (Hanz et al., 2003, Perry, Doron-Mandel et al., 2012). Other key players of the injury retrograde signaling cascade have also been identified. Axonal translation of RanBP1 following injury is required for the interaction between the newly synthesized Importin β1 and Importin α3 protein (Yudin et al., 2008). The Importin β/α complex is then retrogradely transported by dynein motor proteins to deliver the injury signal to the cell body. In addition to the Importin complex, several transcription factors are also locally synthesized and retrogradely transported in response to injury. Key among these are the transcription factor STAT3, which appears to be a necessary survival signal (Ben-Yaakov et al., 2012), and PPARγ, which is

critical for axon regeneration (Lezana, Dagan et al., 2016). Taken together, these studies highlight the important role that axonal translation plays in the axonal injury response, and suggests that variable ability to utilize these mechanisms as part of an injury response underlays differences in regenerative capacity.

Overcoming the challenges of regenerating axons in the CNS may be mitigated by encouraging some of the same local translation seen in peripheral neurons. Many of the challenges faced in axon regeneration as driven by local translation in CNS neurons are the variety of extracellular growth inhibiting molecules present in the spinal column and brain. However, these neurons are capable of local synthesis and conduct it as part of normal physiology. Removing these inhibitory molecules and priming the axonal space for local synthesis has been shown to increase the likelihood of regrowth (Jung, Yoon et al., 2012, Kalinski, Sachdeva et al., 2015, Mar, Bonni et al., 2014, Verma et al., 2005).

Local protein synthesis in the axon provides vital information transfer, structural components, and a degree of cell-body autonomy. The ability to produce proteins on demand, both in physical and temporal space, is a defining functional feature of neurons. Using this ability as criterion for “neuronal status” it is interesting to note that neuronal cells derived from human iPSCs show a transcriptome and proteome in the axon that is strikingly similar to developmentally matched rodent neurons (Bigler, Kamande et al., 2017). Perhaps further work will demonstrate that these similarities lead to equivalent functioning.

Axonal translation in disease

With the accumulating recognition of the complexity of the axonal transcriptome and the roles that these locally synthesized proteins play both in development and in proper function of the mature nervous system, there is now increasing interest in the functional consequences when this process goes awry. Recent years have seen emerging evidence that aberrant axonal localization of mRNAs and disruption of translation can lead to clinical pathology (Table 1). The diseases where disruption of axonal translation have been implicated span the spectrum from diseases of development to those of aging.

Deficits in local translation appear to underlie Fragile X Mental Retardation (Bear, Dolen et al., 2008), and the fragile X mental retardation protein (FMRP) is found within dendritic spines as well as axons and growth cones. It has a well-established role in regulating plasticity in dendritic spines (Bassell & Warren, 2008); however, its role in the axon is less clear. It appears to regulate the axonal proteome (Akins, Leblanc et al., 2012), and loss of this protein decreases synaptic connectivity (Hanson & Madison, 2007). Axonal translational machinery is associated with FMRP in granules, and these Fragile X granules are present not only in hippocampal axons of adult rats, but have also been detected in aged humans (Akins, Berk-Rauch et al., 2017). Loss of FMRP in mouse models has demonstrated that this protein is not required for axonal transport of translational machinery such as ribosomes, or for FMRP-target mRNAs, however it is required to regulate axonal protein synthesis (Akins et al., 2017). Disruption of translational machinery regulation due to the loss or mutation of FMRP may play a causative role in the developmental symptoms, including learning disabilities and cognitive impairment.

A significant number of nuclear-encoded mitochondrial mRNAs are localized within axons, and accumulating evidence implicates disruption in their transport and/or local translation with clinical phenotypes. Loss of local translation of these mRNAs results in diminished mitochondrial health (Aschrafi, Natera-Naranjo et al., 2010, Natera-Naranjo, Kar et al., 2012), and given the importance of mitochondrial health in axonal function it is not surprising that this might result in dysfunction that rises to the level of clinical pathology. For example, when COXIV mRNA is prevented from localizing into axons of neurons in the mouse forebrain, there is an increase in ROS in the cortex. Interestingly, this increase correlates with an anxiety-like and depression-like phenotype that is reminiscent of neuropsychiatric disease in humans (Kar, Sun et al., 2014). Numerous studies have implicated impaired mitochondrial function and increased ROS in neuropsychiatric disease in humans (Manji, Kato et al., 2012). These results suggest that disruption of local translation of nuclear-encoded mitochondrial mRNAs within axons may be an important contributor to these disease processes.

COXIV is not the only axonally-enriched nuclear-encoded mitochondrial mRNA that has been implicated in neuropsychiatric disease. Disruption in nuclear processing of the pre-mRNA of ATP5G1, which encodes a subunit of mitochondrial ATP synthase, may be involved in the intellectual disability associated with mutation in the polyadenosine RNA-binding protein, Zinc Finger Cys3His Protein 14 (ZC3H14) (Kelly, Leung et al., 2014, Pak, Garshasbi et al., 2011, Wigington, Morris et al., 2016). This disruption in nuclear pre-mRNA processing may result in loss of axonal trafficking of ATP5G1. Dysregulation of microRNAs that regulate these nuclear-encoded mitochondrial mRNAs also have been linked to neuropsychiatric disorders. For example, deletions within 22q11 are implicated in schizophrenia. One of the deleted genes in this region encodes Dgcr8, a component of the complex needed to process mature microRNAs. This is associated with reduction in miR-338-3p, which is known to target both COXIV and ATP5G1 (Aschrafi et al., 2010, Chun, Du et al., 2017, Vargas, Kar et al., 2016). Taken together, these results support that aberrant processing of nuclear-encoded mitochondrial mRNAs that are normally enriched in axons, results in reduction in axonal synthesis of these proteins and contributes to neuropsychiatric disorders (Gale, Aschrafi et al., 2017, Kos, Klein-Gunnewiek et al., 2017).

One of the more surprising diseases where changes in axonal protein synthesis have been implicated is Alzheimer's disease (AD). In a recent study, the axonal compartment of primary hippocampal neurons grown in microfluidic chambers were exposed to oligomeric $A\beta_{1–42}$ peptide, resulting in the selective increase of a subset of axonal mRNAs (Baleriola, Walker et al., 2014). This recruitment was followed by a subsequent increase in axonal translation of these recruited mRNAs. Among the mRNAs recruited are those whose protein products are able to modify tau protein or alter amyloid production, both of which are hallmarks of AD pathology. Interestingly, the mRNA encoding the transcription factor ATF4 was also recruited and synthesized in response to $A\beta_{1–42}$ peptide exposure. This locally synthesized ATF4 was then retrogradely transported to the cell body and imported into the nucleus, where it altered transcription. This altered transcription appears to launch a cell death program by triggering increased transcription of the C/EBP homologous protein (CHOP), resulting in death of the neuron. In this way, we see a similar axon to cell body communication mechanism utilizing transcription factors as in the injury-induced signal

comprising STAT3 and PPARγ; however, instead of signaling the cell body to mount a regenerative response the result is a death signal.

Given the importance of RBPs to the proper axonal localization of mRNAs, it is perhaps not surprising that mutations or deletions of RBPs have been implicated in neurological diseases (Liu-Yesucevitz, Bassell et al., 2011). Spinal muscular atrophy (SMA) is an autosomal disease characterized by spinal motor neuron death and skeletal muscle paralysis, and is caused by deletion or mutation(s) of the survival motor neuron 1 (SMN1) gene. SMN has critical housekeeping functions within all cells since it is a necessary component in the assembly of the small nuclear RNP (snRNP) needed for nuclear RNA splicing. Curiously, while SMN is required for this process in all cells, it is motor neurons that selectively die in SMA. Given this, SMN has been proposed to exert a neuron-specific role to account for the selective motor neuron death. A clue to its role comes from studies that identify SMN protein localizing within axons in close association to other RBPs and to mRNAs (Akten, Kye et al., 2011, Dombert, Sivadasan et al., 2014, Fallini, Zhang et al., 2011, Rossoll, Kroning et al., 2002), suggesting a role for SMN in the assembly of the complexes necessary for either the transport, the stability, or the local translation of axonal mRNAs. Further supporting this idea, SMN reduction leads to reduced levels of several mRNAs in axons (Fallini, Donlin-Asp et al., 2016, Rage, Boulisfane et al., 2013, Saal, Briese et al., 2014). Recent studies have identified an axonal localization element within the 3′UTR of the Annexin A2 mRNA, which shows SMN-dependent axonal localization (Rihan, Antoine et al., 2017). Annexin A2 is an actin-interacting protein that plays a role in maintaining the plasticity of the actin cytoskeleton. SMN deficiency has been shown to lead to defects in both microtubule and actin organization, potentially linking the loss of SMN-dependent Annexin A2 mRNA axonal localization and subsequent local translation to SMA pathology. Annexin A2 mRNA is only one of a number of SMN associated mRNAs found in the axon, and it seems likely that disruption in axonal localization of additional mRNAs as a result of loss of SMN protein may play a role in the selective vulnerability of motor neurons.

SMA is not the only motor neuron disease where disruption in RBP-dependent axonal localization of mRNAs has been implicated as contributing to pathogenesis. Mutations in fused in sarcoma/translocated in liposarcoma (FUS/TLS) and Tar DNA binding protein-43 (TDP-43) proteins lead to both familial cases of amyotrophic lateral sclerosis (ALS) and to frontotemporal dementia (FTD). Mutations in TDP-43 decrease the transport of the low molecular weight neurofilament mRNA, likely as a result of decreased anterograde mobility of TDP-43 in axons (Alami et al., 2014). This is not the only mRNA that shows changes in axon levels as a result of TDP-43 mutations. In a recent study, RNA-Seq of mutant TDP-43 (TDP-43^{A315T}) motor neuron axonal samples identified numerous dysregulated mRNAs. Similar to changes seen when SMN protein is lost, TDP-43 mutation driven alterations in the axonal transcriptome resulted in depletion of mRNAs encoding proteins involved in synapse assembly and axon extension (Rotem, Magen et al., 2017). This similarity in axonal transcriptome changes mirrors the similarity in disease pathology between SMA and ALS. Given this parity, it is not surprising then that recent studies also point to changes in axonal mRNAs as a result of FUS/TLS mutations (Scekic-Zahirovic, Sendscheid et al., 2016, Shiihashi, Ito et al., 2016). This mechanism was first supported by studies in fibroblasts, where FUS/TLS was shown to regulate translation of adenomatous polyposis complex

(APC) in specific subcellular domains (Yasuda, Zhang et al., 2013). APC binds to several mRNAs that localize to growth cones (Preitner, Quan et al., 2014), and mutant FUS/TLS results in decreased axonal levels of these mRNAs (Yasuda, Clatterbuck-Soper et al., 2017). Adding to the complexity of the mechanism of pathogenesis in these motor neuron diseases, FUS/TLS has been shown to interact with SMN protein, which may in turn alter SMN's interaction with its mRNAs (Sun, Ling et al., 2015). Finally, the repeat expansion in the C9orf72 gene, which is seen in a significant percentage of familial ALS patients, implicates a non-RBP mediated role for disruption in local translation as a contributing factor in motor neuron disease. The C9orf72 expansion causes disruption in the function of the nuclear pore in neurons, which has been hypothesized to inhibit the transcription response to the Importin α/β retrograde injury signal (Donnelly, Zhang et al., 2013, Haeusler, Donnelly et al., 2014, Zhang, Donnelly et al., 2015). Taken together, there may be multiple points along the axonal mRNA transport-axonal translation spectrum where disruption results in neuronal pathogenesis.

Conclusions

The concept of axonal protein synthesis, while taking many years to gain significant traction, is now in a period of rapid advancement. The advent of new methodologies for isolating purified axonal material and sensitive means of assessing their content, have allowed for identification of the axonal transcriptome in a variety of neuronal types, both during development and into adulthood. With these profiles has come the realization that the axonal transcriptome is highly complex, and that axonal translation has the potential to contribute to a wide variety of processes critical for normal neuronal function. This will likely lead to identification of novel functions for axonal translation, beyond those already established for axon growth and regeneration. The breadth of this population, and the mechanisms used to transport these mRNAs into axons and facilitate their local translation has also lead to the understanding that with such a complex and highly regulated process there is likely to be significant consequences when there is disruption. Emerging studies indicate that multiple steps along the continuum of mRNA processing, transport, local synthesis, and retrograde signaling can be disrupted, resulting in neurodevelopmental and neurodegenerative disease (Figure 1). The specific disease-associated examples discussed above are likely to be only a small fraction of the possible ways in which axonal translation contributes to neuronal disease, and it remains to be seen whether a better understanding of how axonal translation is regulated may lead to potential therapeutic strategies leveraging this knowledge.

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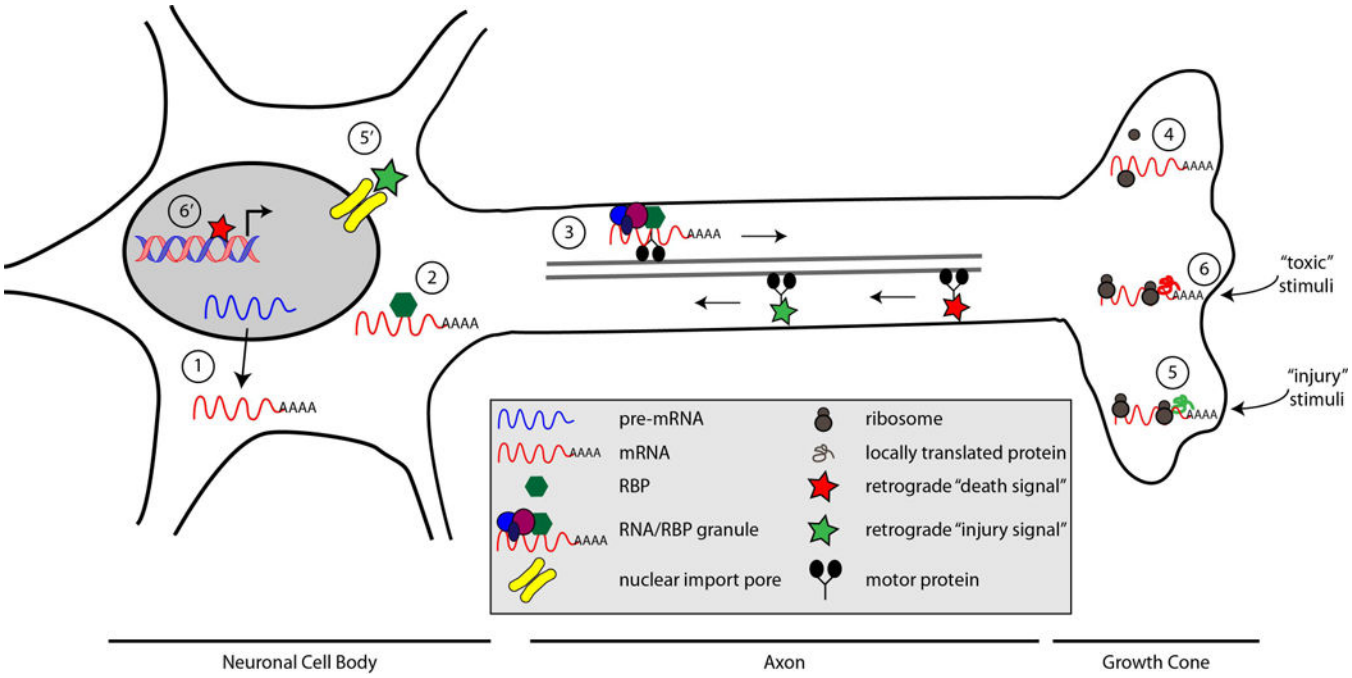

Figure 1. Multiple sites along the axonal protein synthesis continuum have been implicated in neurological disease

(1) Aberrant mRNA processing of nuclear-encoded mitochondrial mRNAs that are normally enriched in axons can result in reduction in axonal synthesis of these proteins and contributes to neuropsychiatric disorders. **(2)** Changes in RBP binding, as a result of loss of the RBP, mutation of the RBP, or loss of the mRNA targeting element have been implicated in SMA and ALS. **(3)** Changes in the rate of anterograde transport, as in Fragile X, or in the amount of anterograde transport, as in ALS causing TDP-43 mutations, can decrease the amount of mRNA available for local translation in the axon. **(4)** Disruption of translational machinery regulation in the absence of FMRP reduces axonal translation. **(5)** Injury signal normally result in local translation and retrograde transport of an injury signal complex. Repeat expansion of C9orf72, a cause of familiar ALS, prevents nuclear import of the injury signal (**5′**), potentially preventing the injury transcription response. **(6)** Exposure to toxic stimuli, such as Aβ peptide, leads to axonal synthesis of ATF4, which is retrogradely transported to the nucleus where it increases transcription of CHOP (**6′**), resulting in neuronal death.

Table 1

Neurodevelopmental and neurodegenerative diseases associated with axonal translation.

Disease	Mechanism of disruption	Reference
Fragile X	Dysregulation of translational machinery due to loss/mutation of FMRP.	Akins et al., 2012 Akins et al., 2017
Psychiatric Disorders	Loss of nuclear-encoded mitochondrial mRNA translation.	Aschrafi et al., 2010 Kar et al, 2014
Alzheimer's Disease	Axonal translation and retrograde transport of transcription factors in response to Aβ peptide.	Baleriola et al., 2014
Spinal Muscular Atrophy (SMA)	Decrease in axonal levels of SMN-associate mRNAs.	Rage et al., 2013 Saal et al., 2014 Fallini et al., 2016
Amyotrophic lateral sclerosis (ALS)	Decreased transport and altered localization of TDP-43 or FUS/TLS-associated mRNAs.	Alami et al, 2014 Rotem et al., 2017 Yasuda et al., 2017
	Disruption of nuclear import caused by C9orf72 expansion	Donnelly et al., 2013 Zhang et al., 2015