PERSPECTIVE

Old dogs with new tricks: intra-axonal translation of nuclear proteins

Many different types of polarized eukaryotic cells have been shown to segregate synthesis for some protein subpopulations to cytoplasmic domains distant from their nucleus. For neurons, these distances can be tens-to-thousands fold more than the diameter of the cell body. Both axons and dendrites make use of this localized protein synthesis to bring autonomy to these far reaches of the cytoplasm (Gomes et al., 2014). This local mRNA translation is often used to mount a rapid response to extracellular stimuli encountered by the distal axon and dendrite. Indeed, activating translation of mRNAs residing locally at the synapse or growth cone brings a much more rapid response than could be achieved by transporting new proteins from the cell body. The neuron likely reaps a cost benefit from this mechanism in terms of energy consumption, since multiple protein copies can be generated from a single mRNA through sequential rounds of translation. Localized protein synthesis could also more effectively position a protein near its site of action or even bring an unanticipated novel function to the protein.

Although many of the proteins synthesized in axons and dendrites act locally within these processes, several lines of evidence indicate that some of these proteins can function in axon-to-soma and dendrite-to-soma communication. This was initially documented for axons with translation of Importin β1 mRNA in peripheral nerves; the encoded Importin β1 protein forms an obligate heterodimer with axonal Importin α protein to ferry signaling complexes from the injured axon to the cell body (Hanz et al., 2003). Subsequent studies have shown that mRNAs for some transcription factors also localize into neuronal processes, with their protein products being retrogradely transported back to the nucleus for stimulus dependent gene regulation. Signal transducer and activator of transcription-3 alpha (Stat3α), cAMP response element-binding protein (CREB), and SMAD proteins are examples of locally synthesized transcription factors (Ji and Jaffrey, 2014). Typically, the neuron already has a pool of these transcription factors residing in or much closer to the nucleus that can be activated through post-translational modification(s). Thus, the reason for locally translating these (and potentially other) transcription factors is not clear. A logical assumption is that the locally generated transcription factor confers a signal that is qualitatively or quantitatively distinct from the proteins residing in the cell body. Recent papers add the chromatin interacting High Mobility Group (HMG) proteins, HMGN5 and HMGB1 or amphoterin, to the now increasing list of nuclear proteins that can be synthesized in neuronal processes (Merianda et al., 2015; Moretti et al., 2015).

Non-biased RNA profiling approaches have shown that hundreds-to-thousands of different mRNAs localize into the processes of cultured neurons (see Minis et al., 2014 and references within). These localized mRNAs include many encoding proteins that were thought to selectively reside and function within the nucleus. The Pertz lab very recently identified HMGN5 mRNA in growth cones of differentiated N1E-115 cells, a neuroblastoma cell line that can be induced into a neuron-like phenotype with neurites extending many microns in length (Moretti et al., 2015). Similar to primary neurons, the neurites of differentiated N1E-115 cells contain mRNAs and synthesize proteins. Moretti et al. (2015) showed that HMGN5 mRNA localizes through its 3′ untranslated region (UTR) in both differentiated N1E-115 cells and primary hippocampal neurons. The locally synthesized HMGN5 protein is actively transported to the nucleus where it assumes a "classic" HMG protein function by modulating chromatin structure. Overexpression of a neurite-targeted, but not a cell body-restricted, HMGN5 mRNA increases neurite outgrowth, and this function requires that the encoded protein interacts with chromatin. Although the retrograde transport of locally synthesized HMGN5 protein provides a mechanism for modulating gene expression, this raises the question of how the locally synthesized HMGN5 functions distinctly from HMGN5 that takes the more direct route by nuclear import following its mRNA translation in the cell body. Somehow the neuron must distinguish between these two sources of HMGN5 protein.

Neurons also have the ability to distinguish between different sources of proteins that function outside of the nucleus. For example, only a small fraction of axonal β-actin protein in axons is synthesized locally, so the bulk of this protein is transported down the axon from the cell body (see Donnelly et al., 2013 and references within). Although the axon has a dynamic pool of actin protein to draw from, the locally synthesized β-actin protein seems to have unique roles in polarized cell migration and axon branching (Shestakova et al., 2001; Donnelly et al., 2013). Similar to HMGN5, we recently showed that HMGB1/amphoterin protein is synthesized in axons, but this axonally generated HMG protein supports neurite growth locally (Merianda et al., 2015). HMGB1/amphoterin mRNA is translationally regulated in axons after axotomy providing a mechanism to locally introduce more HMGB1/amphoterin protein into the axon. This regulation is also linked to the mRNA's UTRs. Surprisingly, axotomy also triggers the existing nuclear HMGB1/amphoterin protein to shuttle out of the nucleus. Such a shift in nuclear HMGB1/amphoterin protein has been demonstrated for several different cell types, with the protein being actively released by some cell types and passively released by necrotic cells to stimulate an inflammatory response (Lotze and Tracey, 2005). Pro-inflammatory actions of HMGB1/amphoterin seem to work through binding to toll-like receptors (Lotze and Tracey, 2005), and inflammation in the dorsal root ganglion can be supportive for regeneration after peripheral nerve injury (Niemi et al., 2013). On the other hand, exogenous HMGB1/amphoterin has been shown to stimulate neurite outgrowth, and expression of the Gecko and

Zebrafish HMGB1/amphoterin orthologs after spinal cord injury supports axon regeneration similar to what we see with the axonally generated mammalian HMGB1/amphoterin protein (Dong et al., 2013; Fang et al., 2014). Although we did not distinguish receptor interactions for the cell body *vs.* axonally synthesized HMGB1/amphoterin, growth-promoting activities of HMGB1/amphoterin have been linked to binding to the receptor for advanced glycation end products (RAGE) (see Merianda et al., 2015 and references within). It is appealing to hypothesize that the spatially and temporally segregated sources of neuronal HMGB1/amphoterin protein establish different functions for the encoded protein cohorts. The distinct regulation for HMGN5 and HMGB1/amphoterin mRNA pools as well as their encoded proteins' functions exemplify some of the questions that remain unanswered for neuronal mRNA localization and localized translation.

How does the neuron regulate transport and translation of the HMGN5 and HMGB1/amphoterin mRNA pools? It is clear from other studies that the UTRs of mRNAs contain *cis*-elements recognized by mRNA binding proteins (RBPs) needed for their transport and/or translational regulation. Both transport and translation of axonal and dendritic mRNAs has been shown to be regulated by extracellular stimuli (Gomes et al., 2014). Transport of HMGN5 and HMGB1/amphoterin is driven by their 3′UTRs. HMGB1/amphoterin mRNA shows constitutive transport into axons and regulation of its localized translation. This suggests that HMGB1/amphoterin mRNA arrives in the axons and is stored until its translation is activated by a stimulus, similar to Importin β1 and other axotomy-induced axonal mRNAs (Gomes et al., 2014). However, HMGB1/amphoterin mRNA shows sustained translational upregulation and it is not clear what mechanisms distinguish its regulation from that of other axotomy-induced transcripts (Merianda et al., 2015). On the other hand, HMGN5 mRNA shows increased transport into growing neurites (Moretti et al., 2015); it is not clear if the HMGN5 mRNA is translated immediately after delivery to neurites or what stimulates the mRNA's transport into neurites. It will be quite interesting to uncover which RBPs regulate transport and translation of neuronal HMGN5 and HMGB1/amphoterin mRNAs (**Figure 1**). Increasing evidence points to cohorts of mRNAs sharing RBPs (see Gomes et al., 2014 and references within), so it is likely that other localizing transcripts will have similar temporal and spatial regulation as HMGN5 and HMGB1/amphoterin mRNAs.

How does the neuron distinguish the locally translated protein from that translated in the cell body? Targeting the mRNA for localized synthesis clearly provides a means to increase the functional repertoire of proteins that could be derived from a single spatial population of an mRNA, but it remains to be determined how the neuron, or any cell for that matter, decodes such information at the protein level. Proteins can assume different functions through protein-protein interactions, so the proteins encoded by localized mRNAs may acquire different functionality from the cell body-encoded proteins by virtue of spatial and temporal contexts of their synthesis (**Figure 1**). Differential post-translational modifications could also help distinguish neurite- from cell body-encoded proteins. Indeed, Moretti et al. (2015) showed preferential phosphorylation of the locally synthesized HMGN5 protein that correlated with the unique chromatin modifying function(s) of the locally synthesized HMGN5. Regardless of mechanism, targeting the mRNA to subcellular domains away from the cell body (or juxta-nuclear region for less polarized cells) can undoubtedly be considered as an initiating event for this functional segregation. Targeting HMGN5 and HMGB1/amphoterin mRNAs into neurites may also be used as a means to overcome their encoded nuclear localization signals by segregating a subpopulation of these mRNAs away from the peri-nuclear region and ensuring two functionally distinct protein populations. With this in mind, the interaction of RBPs with the *cis*-elements that are used to target HMGN5 and HMGB1/amphoterin mRNAs into axons and dendrites may represent the precipitating event that contextually defines the functions of proteins generated by the localized mRNAs.

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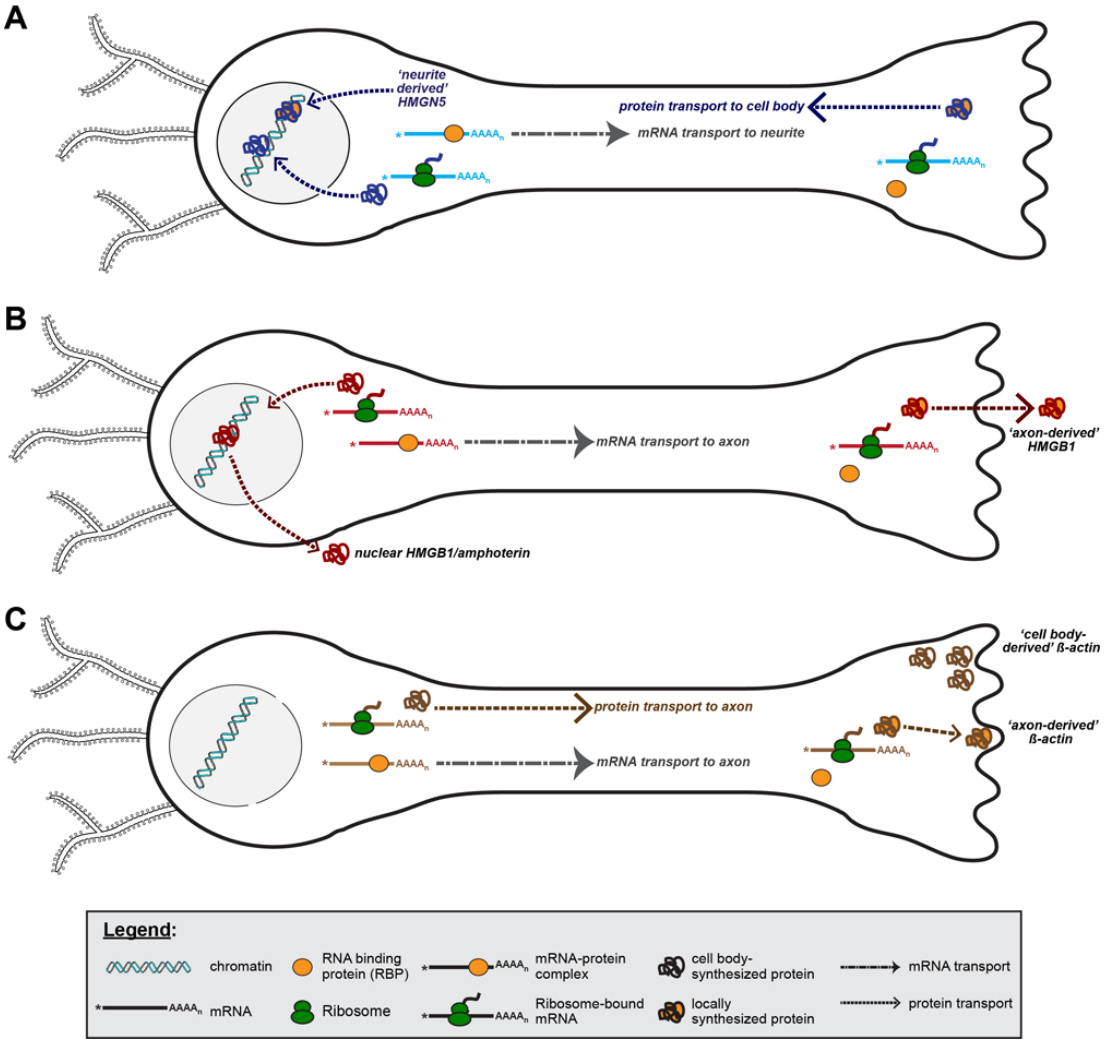

Figure 1 Generating distinct functional protein classes through mRNA targeting.
Schematic illustrates examples of distinct functions for locally synthesized *vs.* cell body-synthesized proteins, with legend indicating the symbol usage. (A) HMGN5 protein generated from its localized mRNA in neurites is retrogradely transported to the cell body triggering different functional outcomes than the HMGN5 protein generated from the cell body, with both acting as chromatin interacting proteins in the nucleus (Moretti et al., 2015). (B) HMGB1/Amphoterin protein that is generated from axonal mRNA does not appear to be retrogradely transported acts locally to increase axon growth, possibly through autocrine or paracrine mechanisms (Merianda et al., 2015). Nuclear HMGB1/amphoterin protein (generated from translation of cell body-restricted mRNA) exits the nucleus after injury and is likely secreted; such injury-induced release of nuclear HMGB1/amphoterin has been associated with pro-inflammatory responses. (C) Axonal β-Actin protein comes both from localized translation of axonal β-actin mRNA and anterograde transport of β-actin protein derived from mRNA translation in the cell body. The axonally generated β-Actin protein appears to take on different functions than that derived from anterograde transport (Donnelly et al., 2013). For each of the examples in A–C, localization of the mRNA to distal neurites is critical for spatially segregating the sources of these proteins. These localizations are driven by mRNA binding proteins (RBPs) binding to the mRNAs in cell body. HMG: High mobility group.

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