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Perspective

The Central Dogma Decentralized: New Perspectives on RNA Function and Local Translation in Neurons

Christine E. Holt[1,*] and Erin M. Schuman[2,*]
[1]Department of Physiology Development and Neuroscience, Anatomy Building, Downing Street, University of Cambridge, Cambridge CB2 3DY, UK
[2]Max Planck Institute for Brain Research, Max von Laue Strasse 4, 60438 Frankfurt am Main, Germany
*Correspondence: ceh33@cam.ac.uk (C.E.H.), erin.schuman@brain.mpg.de (E.M.S.)
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The elaborate morphology of neurons together with the information processing that occurs in remote dendritic and axonal compartments makes the use of decentralized cell biological machines necessary. Recent years have witnessed a revolution in our understanding of signaling in neuronal compartments and the manifold functions of a variety of RNA molecules that regulate protein translation and other cellular functions. Here we discuss the view that mRNA localization and RNA-regulated and localized translation underlie many fundamental neuronal processes and highlight key issues for future experiments.

Background

It is now clear that individual neurons are highly compartmentalized with specific functions and/or signaling that occur in restricted subcellular domains. Extrinsic signals are often spatially localized such that they are "seen" by restricted parts of a neuron, such as synaptic input to a specific dendritic spine or a guidance cue encountered by a growth cone. Twenty-five years ago, when the first issue of *Neuron* was published, it was well appreciated that the neurons were capable of local information processing, but the potential cellular mechanisms that established and regulated local compartments were not well understood. Dendritic spines had been proposed as biochemical and/or electrical compartments (Harris and Kater, 1994; Koch and Zador, 1993), and polyribosomes had been identified at the base of spines (Steward and Levy, 1982). However, the view that dominated until nearly the end of the twentieth century was that the central dogma (DNA-RNA-protein) was carried out centrally—in the nuclei and somata of neurons. In that context, the localization of mRNA observed in some cells was thought to represent a specialized mechanism that operated in unique biological systems, such as egg cells, where storage of mRNAs is needed for subsequent patterning of the early embryo (see Martin and Ephrussi, 2009 for review). Evidence from a number of studies in the last decade, particularly in neurons, has led to a revolution in our thinking. Although the field is still young, it is becoming clear that RNA-based mechanisms provide a highly adaptable link between extrinsic signals in the environment and the functional responses of a neuron or parts of a neuron. This is accomplished by the localization of both protein-coding and noncoding RNA in neuronal processes and the subsequent regulated local translation of mRNA into protein. Here we discuss some of the key findings that lead us to the view that mRNA localization and RNA-regulated and localized translation underlie many fundamental cellular processes that are regulated by extrinsic signals in neurons, such as memory, dendrite and arbor branching, synapse formation, axon steering, survival, and likely proteostasis.

The dynamic regulation of protein synthesis is essential for all cells, including neurons. Over 50 years ago, in vivo experiments (in a variety of species) established a clear functional link between protein synthesis and long-term memory (see Davis and Squire, 1984 for review), indicating that proteome remodeling underlies behavioral plasticity. These observations were paralleled by in vitro studies of synaptic plasticity demonstrating a clear requirement for newly synthesized proteins in the long-term modification of synaptic function (see Sutton and Schuman, 2006 for review; also, Tanaka et al., 2008). This link between protein synthesis and long-term plasticity is most recently reinforced by studies showing that targeted genetic disruption of signaling molecules that regulate protein translation interfere with long-term synaptic or behavioral memories (Costa-Mattioli et al., 2009). The above studies, while indicating a requirement for protein synthesis, do not address the location. We now know dendrites and axons of neurons represent specialized cellular "outposts" that can function with a high degree of autonomy at long distances from the soma, as illustrated by the remarkable ability of growing axons to navigate correctly after soma removal (Harris et al., 1987) or isolated synapses to undergo plasticity (Kang and Schuman, 1996; Vickers et al., 2005). The identification of polyribosomes at the base or in spines (Steward and Levy, 1982) together with metabolic labeling experiments that provided the first evidence of de novo synthesis of specific proteins in axons and dendrites (Feig and Lipton, 1993; Giuditta et al., 1968; Koenig, 1967; Torre and Steward, 1992) indicated the competence of these compartments for translation. Subsequent studies demonstrated that specific subsets of mRNAs localize to synaptic sites (Steward et al., 1998) and directly linked synaptic plasticity with local translation in dendrites (Aakalu et al., 2001; Huber et al., 2000; Kang and Schuman, 1996; Martin et al., 1997; Vickers et al., 2005), providing definitive proof that dendrites are a source of protein during plasticity.

In axons, the idea of local protein synthesis has been slower to find acceptance, no doubt hindered by the classical view of axons as information transmitters rather than receivers; so, why would local protein synthesis be required? Although ribosomes were identified in growth cones in early ultrastructural studies (Bunge, 1973; Tennyson, 1970), they were rarely observed in adult axons. It is now thought that at least part of the explanation for their apparent paucity lies in their localization close to the plasma membrane in axons (Sotelo-Silveira et al., 2008) where ribosomal subunits can associate directly with surface receptors (Tcherkezian et al., 2010). In addition, evidence indicates that myelinated axons can tap into an external supply of ribosomes by the translocation of ribosomal proteins from Schwann cells (Court et al., 2011). Growing and navigating axons are clearly information receivers, like dendrites, since their growth cones steer using extrinsic signals. Indeed, the first functional evidence for local protein synthesis in axons came from studies that showed that cue-induced directional steering is abolished by inhibitors of protein synthesis, including rapamycin, in surgically isolated axons (Campbell and Holt, 2001). Subsequent studies confirmed this result in different neurons (Wu et al., 2005; Yao et al., 2006) and revealed that local protein synthesis underlies growth-cone adaptation, gradient sensing, and directional turning in growing axons (Leung et al., 2006; Ming et al., 2002; Piper et al., 2005; Yao et al., 2006). In addition, axonal protein synthesis is elicited in response to injury and plays key roles in axon regeneration and maintenance (Jung et al., 2012; Perry et al., 2012; Verma et al., 2005; Yoon et al., 2012; Zheng et al., 2001).

Compartments

Neuronal function is highly dependent on spatially precise signaling. Increasing evidence indicates that the complex morphology of neurons has created biological compartments that subdivide the neuron into spatially distinct signaling domains important for neuronal function (Hanus and Schuman, 2013). Dendritic spines represent a specialized (“classical”) cellular compartment in which subsets of specific proteins (e.g. receptors, channels, signaling molecules, and scaffolds) are collected together with a common function for receiving and processing electrical and chemical input. Spines have a distinct structural morphology and, as such, are easy to classify as a compartment. Although spines are small (~1 μm^3), they can still be subdivided into further functional compartments (see Chen and Sabatini, 2012 for review) with multiple microdomains, raising the question of how a compartment is defined. For example, a recent superresolution imaging study demonstrated that, within synapses, AMPA receptors are clustered into small nanodomains (~70 nm in diameter) that contain on average ~20 receptors (Nair et al., 2013). These nanodomains are dynamic in both their shape and position and may have a limited lifetime. Anatomically and functionally distinct compartments also exist in axons, such as the growth cone, the axon initial segment, and terminal arbor. Equally, there are examples of compartments that exhibit no obvious “anatomical” specializations. In axons, for example, some membrane proteins are localized to restricted segments of the axon (Fasciclins, Tag1/L1, Robo) (Bastiani et al., 1987; Dodd et al., 1988; Katsuki et al., 2009; Rajagopalan et al., 2000) indicative of plasma-membrane compartmentalization. In addition, second-messenger signaling molecules such as calcium and cyclic nucleotides, once thought to signal extensively throughout a cell, are now known to be highly regulated such that increases in concentration can be confined to a small space, creating a signaling compartment. Selective activation of a single spine on a dendrite, for example, can provide the receiving neuron with information about a specific stimulus (Varga et al., 2011). Compartments may be overlapping or distinct and range in size depending on the biological function. Ultimately, a neuron must integrate the information received from multiple compartments. As such, future experiments aimed at understanding how different compartments emerge and what mechanisms generate such spatially precise intracellular patterning will be very informative.

Compartmentalized signaling presents several challenges to the cell, a prime one being the localization of its component parts. Specific molecules must be transported and delivered to the appropriate subcellular destinations. One of the remarkable features of RNA is its ability to be spatially localized and, therefore, potentially contribute to neuronal compartmentalization. Historically, localized mRNAs have been studied during development (see Martin and Ephrussi, 2009). That localized RNA is more often the rule than the exception is spectacularly illustrated by the finding that 71% of the *Drosophila* embryo transcriptome is localized to specific subcellular compartments (Lécuyer et al., 2007). The proteins encoded by localized mRNAs are also concentrated at the site suggesting that mRNA localization and the ensuing local translation plays an important role in positioning proteins for cellular functions.

Asymmetry and Spatial Signaling

A general function of mRNA localization is the generation of asymmetry. mRNAs tend to be abundantly localized to the peripheral domains and motile parts of neurons where they are optimally positioned for the arrival of external signals, e.g., in dendrites (synaptic activation) and growth cones. Subcellular asymmetry can lead to highly polarized dynamics and cell morphology that can operate on a remarkably fine scale.

Growth-Cone Spatial Signaling

To navigate, growth cones must be able to make directional turns, which demands asymmetry. In retinal growth cones, for example, which are only 5 μm in diameter, a polarized external gradient of netrin-1 triggers increases in both the transport and translation of β-actin mRNA on the gradient near side (Leung et al., 2006; Yao et al., 2006). This polarized translation leads to a rapid (5 min) polarized increase in β-actin protein that helps to drive axon turning towards the gradient source. Interestingly, different cues show specificity in their effects on mRNA transport and translation. Different growth factors, for example, trigger the transport of a specific repertoire of mRNAs in axons (Willis et al., 2005, 2007; Zhang et al., 1999), and different guidance cues elicit the translation of specific subsets of mRNAs (Leung et al., 2006; Piper et al., 2006; Shigeoka et al., 2013; Wu et al., 2005; Yao et al., 2006). β-actin mRNA translation is triggered by netrin-1 but not Sema3A, whereas RhoA and cofilin mRNA translation is induced by Sema3A but not netrin-1. This has given rise to the differential translation model suggesting

that translation-dependent repulsive and attractive turning in growth cones depends on the differential translation of mRNAs involved in assembly or disassembly of the actin cytoskeleton (Lin and Holt, 2007). Several aspects of this translation-driven cue-induced turning remain to be understood, such as how receptor activation signals mRNA recruitment and, critically, how specific subsets of mRNA are translated.

Readout of Spatial Position In Vivo

Navigating growth cones encounter a series of patterned molecular cues along the pathway from which they must read out their spatial position. Although there are several examples of stimulus-induced local translation in axons in vitro (Shigeoka et al., 2013), it has only recently become possible to investigate translation in neuronal compartments in vivo. Early studies by Flanagan and colleagues showing compartmentalized expression of EphA2, recapitulated by a translation reporter, in the post-midline crossing segment of commissural spinal cord axons introduced the idea that the growing tip of the axon is stimulated by a regionally expressed cue (e.g., at the midline) that triggers the region-specific translation of proteins needed for pathfinding (Brittis et al., 2002). A recent study provides direct evidence for this type of mechanism in the control of Robo expression and midline guidance (Colak et al., 2013). Two Robo3 receptor isoforms have opposing roles in guiding axons to and away from the midline, and their expression is compartmentalized in pre-crossing (Robo3.1) and postcrossing (Robo3.2) axonal segments (Chen et al., 2008). The switch to Robo3.2 expression at the midline (the transcript of which contains a premature termination codon) is controlled by midline-induced axonal protein synthesis coupled with nonsense-mediated mRNA decay. This provides an elegant mechanism for turning on synthesis time linked to the crossing event (Colak et al., 2013).

It was not previously technically possible to inhibit translation of a specific transcript in a compartment-specific manner. Recently, however, new tools have been developed that allow separate manipulation of specific neuronal compartments in vivo such as targeted delivery of siRNAs or antisense morpholinos and conditional targeting of 3′UTRs (Perry et al., 2012; Yoon et al., 2012). These subcellular-directed approaches are beginning to yield information suggesting that local translation is involved in regulating multiple aspects of axonal and dendritic biology.

Guidance cues induce immediate steering responses in growth cones via classical signaling pathways that involve receptor activation and phosphorylation of downstream signaling molecules (Bashaw and Klein, 2010). Some of these "immediate" steering responses also involve local translation, as discussed above. Thus, local translation can provide new proteins on demand at subcellular sites for "immediate" use. Interestingly, local translation in response to extrinsic cues has recently been shown to provide proteins for "delayed" use in axon growth and regeneration. Examples of this are the de novo synthesis of proteins that shuttle back to the nucleus where they influence transcriptional output (Cox et al., 2008; Perry et al., 2012), and another is the de novo synthesis of surface receptor proteins that are employed later in a growth cone's journey (Leung et al., 2013).

Spatial Signaling in Dendrites

Recent advances in experimental procedures, allowing the stimulation of individual synapses, have shown that synapses can be independently regulated by synaptic activity (Matsuzaki et al., 2004). On the other hand, other studies emphasize the consideration of the dendritic branch as a computational unit (Govindarajan et al., 2011). Taken together, it seems reasonable to consider a range of spatial domains over which signaling can occur, which would span the scale from subdomains in spines to dendritic branches to the entire neuron. These data can be compared to what we know about the quantitative localization of the protein-synthesis machinery. Indeed, it is clear that many synapses possess a polyribosome nearby (Ostroff et al., 2002). Moreover, recent high-resolution in situ hybridization data suggest that mRNA molecules are distributed in local domains (Cajigas et al., 2012), but not necessarily specific to individual synapses. Preliminary estimates of mRNA numbers indicate that there may not be sufficient copies of individual mRNA species for each synapse to have an exclusive and dedicated molecular toolbox. These data imply that there is local sharing of cell biological machineries, including the machinery for protein synthesis and degradation. It remains unclear, however, over what spatial scale local translation can be regulated and stimulated in dendrites. For example, is stimulation of a single spine sufficient to regulate local translation, and, if so, over what spatial domain do the newly synthesized proteins function?

RNA in Neurons

The past view that RNA acts primarily as an inert intermediate between genes and proteins has undergone a revolution in recent years with discoveries of both new classes of RNAs (e.g., noncoding RNAs, (see Ulitsky and Bartel, 2013 for review) and new RNA-based mechanisms of gene regulation (e.g., microRNA and RNAi silencing) (see McNeill and Van Vactor, 2012 for review). Indeed, given the relatively constrained diversity of proteomes across cells and organisms, RNA-based mechanisms (diverse RNA species and RNA functions) represent a unique platform to diversify and specialize cells, especially neurons. Numerous new roles for RNA have been found in recent years, expanding the role of RNA to controlling many and diverse cellular processes, including stimulus-induced local translation that underlie adaptive responses in neurons (e.g., memory, axon guidance, and maintenance). In addition, RNA's role may not be limited to the cells where it is synthesized, as new studies indicate it can be transferred between cells (via exosomes) (Sharma et al., 2013) and even between organisms (Sarkies and Miska, 2013), bringing a whole new era of RNA function in cellular communication into focus.

mRNA

The demonstrations that local protein translation functions during synaptic development and plasticity led to the hunt for specific mRNAs that could be translated in these local compartments. For many years, in situ hybridization was the method of choice, and several individual mRNAs were visualized in dendrites, including the mRNA for the Ca^{2+}-calmodulin-dependent protein kinase alpha subunit, CaMKIIα (Burgin et al., 1990; Mayford et al., 1996), MAP2 (Garner et al., 1988),

Shank (Böckers et al., 2004), and β-actin (Tiruchinapalli et al., 2003). In growth cones and axons, in situ hybridization provided evidence for several different mRNAs, including β-actin (Bassell et al., 1998; Kaplan et al., 1992; Wu et al., 2005). Recent microarray approaches and deep RNA sequencing have dramatically expanded the local transcriptome in both dendrites and axons (Poon et al., 2006; Zhong et al., 2006). One of the most surprising findings to come out of these studies is the vast number of mRNAs that are present in these neuronal compartments. Growing axons have 1000–4500 mRNAs (Zivraj et al., 2010), while dendrites have >2500 mRNAs (Cajigas et al., 2012). The mRNAs resident in these compartments span many different functional classes of molecules: metabolism, translation, degradation, receptors/channels, cytoskeleton, etc. Many functional categories are shared between the two compartments, although there are numerous distinct compartment-specific subsets of mRNAs, e.g., GAP43 mRNA in axons and neurotransmitter receptor subunits in dendrites.

The localization of mRNA to cellular compartments involves recognition of information that is contained in the 3′ and/or 5′ untranslated (UTR) sequences. The use of mRNA localization to achieve protein localization may arise from the fact that, at least theoretically, unlimited address information can be built into the 3′ and/or 5′ UTRs of mRNA without altering its gene-coding function, whereas there is a tight limit to how much additional coding sequence can be added to a protein without ramifications for function. The family of proteins that bind, transport, localize, and regulate the translation of mRNAs are known as RNA-binding proteins (RBPs) (see Darnell, 2013 for review). RBPs bind to *cis*-elements in the 3′ and 5′ UTRs of mRNAs. RNA-binding proteins complexed with mRNA, other RNA species, and accessory proteins are thought to be assembled in the cell body and form RNA granules (Kiebler and Bassell, 2006). During transport on microtubules and microfilaments to its destination (e.g., Hirokawa, 2006 and Czaplinski and Singer, 2006), the mRNA cargo is thought to be "silenced" by translational repressors (Krichevsky and Kosik, 2001). Once transported, it is unclear how or whether mRNAs are anchored near translational sites—or if they show continued dynamics. Both stationary and anchored particles have been observed in dynamic mRNA imaging experiments (Lionnet et al., 2011).

RNA-binding proteins are an important class of regulatory molecule that recognizes specific nucleotide sequences in RNA (Ray et al., 2013). IP-Seq analysis has revealed, unexpectedly, that some RBPs can bind hundreds of different mRNAs (see Darnell, 2013 for review). Some RBPs, however, appear to be cell-type specific, such as Hermes (RPBMS2) that is expressed exclusively in retinal ganglion cells in the CNS and its knockdown causes severe defects in axon terminal branching (Hörnberg et al., 2013). The number of mRNA-binding proteins identified by known RNA-binding domains is relatively small (around 270) given the increasingly large number of transcripts found in axons and dendrites. Recent work using interactome capture in embryonic stem cells has significantly expanded the number of RBPs, adding a further ~280 proteins to the repertoire, including, remarkably, many enzymes such as E3 ubiquitin ligases with previously unknown RNA-binding function (Kwon et al., 2013). Several RBPs have been implicated in neurological disorders, such as FMRP in Fragile X syndrome and survival of motor neuron protein (SMN) in spinal muscular atrophy (Bear et al., 2008; Liu-Yesucevitz et al., 2011), and translation dysregulation has recently been implicated as a major factor in autism (Gkogkas et al., 2013; Santini et al., 2013).

Noncoding RNAs

In recent years the discovery of noncoding RNAs, including miRNAs (which use sequence complementarity to recognize target mRNA), has revealed unanticipated and enormous potential for the regulation of mRNA stability and translation, as well as other functions. Given the huge and unanticipated number of mRNAs detected in axons and dendrites, it is perhaps not surprising that these noncoding RNAs also exist—and are even enriched—in neuronal compartments. One might even argue the complex morphology and functional specialization of neurons provides a hotbed for mRNA regulation that can potentially be mediated by noncoding RNAs. Indeed, an analysis of 100 different miRNAs discovered the differential distribution of some miRNAs in dendrites versus somata and copy numbers in individual neurons as high as 10,000—equivalent to the number of synapses a typical pyramidal neuron possesses (Kye et al., 2007). Recently, the differential distribution of miRNAs has been also reported in axons versus soma (Natera-Naranjo et al., 2010; Sasaki et al., 2013) and recently emerged as regulators of axon growth and branching (Kaplan et al., 2013). Moreover, the enrichment of miRNAs in synaptosomes isolated from specific brain regions has also been reported (Pichardo-Casas et al., 2012). miRNAs have now been shown to regulate many synaptic functions (see Schratt, 2009 for review). In addition, miRNAs themselves are regulated by behavioral experience (Krol et al., 2010) as well as synaptic plasticity (Park and Tang, 2009). More recently, the appreciation of other types of noncoding RNAs have come into focus, though very little is known about their function in neurons. This includes small-nucleolar RNA-derived and transfer RNA-derived small RNAs, firstly identified as degradation products, and long noncoding RNA known as regulators of gene transcription, that may regulate gene expression posttranscriptionally. A recent study demonstrated, for example, that a long noncoding RNA that is anti-sense to a K^+ channel subunit (Kcna2) is upregulated following peripheral nerve injury, leading to a downregulation of the K^+ channel and a resulting increase in the excitability of DRG neurons, increasing neuropathic pain (Zhao et al., 2013).

Technical Hurdles and Advances

Isolating Compartments

In the early years, the study of local translation was hampered by the technical difficulty of obtaining pure and sufficient quantities of dendrites and axons for analysis. Pioneering studies used metabolic labeling to demonstrate the synthesis of specific proteins such as tubulin in axons (Giuditta et al., 1968; Koenig, 2009), but the possibility that the signal arose from cell-body contamination could not be eliminated due to these technical limitations. Localized translation was convincingly demonstrated by surgically severing the soma from its processes (Aakalu et al., 2001; Campbell and Holt, 2001; Kang and Schuman, 1996) and, more recently, by the use of chambers in which the processes (dendrites or axons) are fluidically isolated from cell bodies

(Eng et al., 1999; Taylor et al., 2010). Other methods for isolating neuronal processes include substrates with limited pore size that allow axons to penetrate but not cell bodies (Torre and Steward, 1992; Zheng et al., 2001) and laser capture microdissection (Zivraj et al., 2010). These methods combined with the rapid increase in the sensitivity of profiling techniques have enabled genome-wide transcriptome analyses to be performed on axons and dendrites in a variety of neurons (see below).

Tagging Newly Synthesized Proteins

The visualization and identification of newly synthesized proteins has also been a hurdle due to issues of sensitivity (detecting low levels of newly synthesized proteins) as well as difficulties in distinguishing between the movement of existing proteins and the synthesis of new proteins. Puromycin, a tRNA analog, can be used together with fluorescent tags (Smith et al., 2005) or antibodies (Schmidt et al., 2009) to label sites of protein synthesis. Fluorescent reporters, such as photo-switchable Kaede, fused to the 3′UTR regulatory region of mRNAs of interest have enabled de novo protein synthesis to be monitored live in neuronal processes (Aakalu et al., 2001; Brittis et al., 2002; Leung et al., 2006). In addition, new methods have been developed to selectively label the pool of newly synthesized proteins, to ascertain a given cell type or cellular compartment as the site of synthesis, and to visualize the newly synthesized proteins. These methods make use of noncanonical amino acids that cross cell membranes and get charged onto tRNAs by the cell's own tRNA synthetases and then incorporated into new protein during protein synthesis. These techniques, bio-orthogonal noncanonical amino acid tagging (BONCAT) and fluorescent noncanonical amino acid tagging (FUNCAT) can be used to selectively identify (Dieterich et al., 2006) or visualize (Dieterich et al., 2010) newly synthesized proteins. A modification of the NCAT method, which in principle enables one to label newly synthesized proteins in specific cell types, has also recently been developed (Ngo et al., 2012), and NCAT can be used in combination with 2D difference gel electrophoresis (DIGE-NCAT) to compare the proteomes of specific subcellular (e.g. axonal) compartments (Yoon et al., 2012).

Looking Ahead

There are many questions for the future, as noted below.

1. How Should We Think about Subcellular Compartments?

We know that some compartments (like spines) have plasma membrane as a boundary that can serve to compartmentalize chemical and electrical signals. Other compartments could be determined by the spatial arrangement of molecules, cytoskeleton, or limited diffusion. Are compartments "static" when bounded by anatomy (e.g., a spine) but dynamic when determined by signaling molecule volumes? What defines a subcellular compartment such that mRNAs contain specific addresses to target them there?

2. How Do mRNAs Reach Neuronal Compartments?

Some mRNAs are targeted specifically to axons and dendrites and even to the growth cone—how is this targeting achieved? While we have in hand several "zip codes," there are certainly many messages for which a clear consensus sequence in the UTR has not emerged. In addition, in some cases the signal for recognition by an RNA-binding protein may reside in the secondary structure of the mRNA, rather than the nucleotide sequence. The fact that current secondary structure prediction techniques are limited to small stretches of nucleotides (~100) complicates our ability to identify binding motifs in 3′UTRs. Adding to the complexity is the recent observation that low-complexity regions of RNA-binding proteins are sufficient to create reversible RNA granule-like structures (Kato et al., 2012). The expanded identification of RBPs as well as the ability to define the binding sites with methods like HITS-CLIP (Licatalosi et al., 2008) should dramatically enhance our knowledge of the binding sites. Future studies should focus on the dynamics of the RNA-protein interactions in cellular contexts. In addition, the possibility that RNA might be delivered from extracellular sources (e.g., via exosomes from neighboring neurons or glia) is a recently suggested exciting idea.

3. How Is the Repertoire of Localized mRNAs Regulated?

Unbiased genome-wide analyses have shown that the mRNA repertoire is dynamically regulated with the mRNA repertoire changing over time (Gumy et al., 2011; Zivraj et al., 2010). In addition, it is clear that synaptic activity can lead to the regulated trafficking of mRNA to the distal processes (e.g., Steward et al., 1998). Is this regulated at the level of transcription, or is there some "gating" mechanism that regulates the trafficking of specific transcripts into dendrites/axons? Evidence with ephrinB in RGCs indicates that although the transcripts are present in somas early in development, they do not move into axons until later, suggesting that some kind of specific gating mechanism may exist.

4. How Many Molecules of mRNA Are in a Compartment?

Currently, little is known about the quantitative aspects of mRNA localization and translation in neurons. For example, how many RNA molecules are needed to provide a functionally significant amount of protein? How many proteins are synthesized from a single mRNA? One might speculate that some classes of proteins, such as cytoskeletal, would be translated much more than others—such as receptors or channels—and transcript abundance could reflect this difference. In theory, just a few new channel or receptor proteins could be sufficient to alter signaling characteristics within a neuronal microdomain. In addition, a low abundant transcript could be stable and translated with high efficiency. Thus, low-abundance transcripts could exert a significant physiological effect and should not be overlooked in profiling analyses. This also raises the intriguing question of whether translation from monosomes, rather than polysomes, may be more common in distal neuronal compartments where there could be demand for a few highly localized proteins. New high-resolution single molecule detection methods (Cajigas et al., 2012; Park et al., 2012) and live-imaging methods for translation (Chao et al., 2012) will be valuable when answering these sorts of questions.

5. What mRNAs Are Translated in Subcellular Compartments In Vivo?

With the advent of TRAP (translating affinity purification) technology (Heiman et al., 2008) it will be possible in the future to answer this question in specific neuronal compartments of specific subsets of neurons. For example, cell-type specific Cre-driver lines can be crossed with the RiboTag mouse (Sanz

et al., 2009), which expresses HA-tagged endogenous ribosomal protein (Rrl22), thereby generating mice with specific neurons expressing HA-tagged ribosomes. These can be isolated from mouse brains by immunoprecipitation at different ages and under different conditions (and diseased), and RNA-Seq analysis can identify the ribosome-protected, and therefore, actively translating transcripts. This will be of huge importance in characterizing and understanding the translatome of neuronal compartments. Thus, current technology now offers the exciting possibility of being able to discover differences in the dendritic or axonal translatome of diseased (e.g., autosomal models) individuals.

6. How Is Translation Regulated in Space?

How does the spatial morphology of the dendrite, axon, or spine contribute to or constrain protein synthesis? It was recently shown that spines enhance the cooperative interaction among multiple inputs (Harnett et al., 2012). These observations suggest that the amplifying and coordinating properties of dendritic spines have an effect on neuronal input processing and may influence information storage by promoting the induction of clustered forms of synaptic and dendritic plasticity among coactive spines. This could allow spines to enhance the ability of neurons to detect, uniquely respond to, and store distinct synaptic input patterns (Harnett et al., 2012). Different patterns of synapse activation can lead to protein synthesis-dependent or -independent plasticity (Govindarajan et al., 2011). However, the importance and mechanism of specific protein translation remains to be examined in this cooperativity. Since there are mRNAs that are differentially distributed in the length of the dendrites, it is tempting to speculate that there is a role for protein synthesis in regulating the functional compartment in dendrites and spines. Thus, while it is clear that protein synthesis occurs in the dendrite and that it is regulated by neuronal activity, the extent to which the activity of single synapses or synaptic regions stimulates protein synthesis, or alters protein localization, remains unknown. Moreover, the importance and impact of synapse location along the dendrite or axon for protein synthesis is unknown.

7. How Is Translation Coordinated with Degradation?

In the small cytoplasmic volume of a dendritic spine or growth cone, there is a limit to the amount of protein that can fit into the space before molecular crowding becomes a problem. While it is clear that changes in synaptic transmission involve extensive regulation of the synaptic proteome via the regulated synthesis and degradation of proteins (Fonseca et al., 2006; Wang et al., 2009), it is not well understood how these two processes are coordinately regulated to achieve the desired level of individual proteins at synapses. Indeed, this is another level of homeostatic control that must exist in order for synapses to maintain the desired level of receptors, scaffolds, and signaling molecules. Changes in the steady-state level of a protein have to be particularly fast and fine-tuned in neurons, due to the fast nature of synaptic transmission and the rapid induction of plasticity.

8. How Specific Is mRNA Translation, and How Is It Regulated?

How are specific mRNAs translated and not others? Studies using either global activity manipulations (TTX/APV) (Sutton et al., 2004) or application of an D1/D5 agonist (Hodas et al., 2012) have suggested large-scale (at least ~100 distinct proteins synthesized) changes in the dendritic proteome. Similarly, global cue stimulation of axons elicits the de novo translation of hundreds of new proteins (Yoon et al., 2012). In these studies, however, the stimulation was applied to the entire network (dish of cultured neurons or brain slice). Under physiological conditions the spatial and temporal profile of synaptic and cue stimulation is on a much finer scale and the translational readout is likely limited. Indeed, we know that different cues can trigger translation of specific subsets of mRNAs in the growth cone (Lin and Holt, 2007). The mechanisms by which specific patterns of synaptic signals (e.g., different frequencies of stimulation, different concentrations or gradients of agonists) and receptor activation lead to activation of the translation machinery are not well understood. Mechanistically, it is clear that elements contained in the 5′ and 3′UTR of mRNAs can regulate their translation initiation. In addition, it is probably the case that the spatial proximity of an mRNA to an active translation site plays a role. The use of high-resolution imaging techniques and focal stimulation should provide answers to these questions.

9. What Roles Do MicroRNA and Other Noncoding RNAs Play in Regulating Local Translation and Neuronal Function?

In neurons, the miRNA function has been explored both individually and on a population level, but a broad conceptual understanding is still lacking. Moreover, if miRNAs regulate mRNA translation and expression in different neuronal compartments, what regulates the expression of miRNA themselves? The accessibility of deep sequencing has enabled the detection of other noncoding RNA species in neurons. These additional RNA classes can directly regulate translation, regulate miRNA function, or serve as scaffolds for other molecules, making the levels of regulation and interactions potentially extremely complicated. In addition, the recent appreciation of the abundance and regulatory potential of other noncoding RNAs, mostly in nonneuronal cell types, adds another level of complexity, including the recent demonstration of regulation by circular RNAs that may serve as either shuttles, assembly factories, or sponges for miRNAs and/or RBPs (Hentze and Preiss, 2013). Based on this, it is likely that a real understanding of the complexity of RNA function in neurons will require not only investigation of individual molecules but also a systems biology perspective where the entire network of RNA molecules and their targets can be considered together (see Peláez and Carthew, 2012).

10. Do Specialized Ribosomes Exist, and Can They Tune Translation?

While ribosomes are readily visible in dendrites spines (Ostroff et al., 2002) and growth cones (Bassell et al., 1998; Bunge, 1973) how they are transported and whether they are sequestered or anchored is not well understood. A mechanism that could provide specificity or docking would be the specialization of ribosomes by accessory proteins or subunits. One of the most intriguing questions raised by recent work is whether ribosomes are tuned to translating specific mRNAs. This possibility is suggested by recent studies showing that haplo-insufficiency of several different ribosomal proteins give rise to specific

phenotypes rather than affecting all cells ubiquitously (Kondrashov et al., 2011; Uechi et al., 2006; Xue and Barna, 2012). This has given rise to the notion of a "ribocode" that suggests heterogeneity in the composition of ribosomes, enabling ribosomes to be tuned to translate specific mRNAs via specific ribosomal proteins (Xue and Barna, 2012). In addition, a striking and curious feature of many recent sequencing studies is the detection of many ribosomal subunits in dendritic or axonal fractions. Indeed, the single most abundant class of mRNAs encode ribosomal proteins in axons (Andreassi et al., 2010; Gumy et al., 2011; Taylor et al., 2009; Zivraj et al., 2010). Thus, an additional intriguing possibility suggested by the abundance of ribosomal protein mRNA in axons and dendrites is that ribosomal proteins may be synthesized de novo. This could provide proteins for in situ repair of ribosomes, or even more interestingly could provide onsite "tuning" of translation (Lee et al., 2013).

11. Does Dysregulated Protein Synthesis Underlie a Wide Range of Neurological Disorders?

One of the most exciting clinically relevant findings to emerge from recent work is the link between dysregulated synaptic protein synthesis and neurological disorders (Bear et al., 2008; Darnell and Klann, 2013; Liu-Yesucevitz et al., 2011). Mouse models of neurodevelopmental disorders such as autism spectrum disorder (ASD) show significant improvement on treatment with reagents that target the protein-synthesis pathway (Bear et al., 2008; Darnell and Klann, 2013; Gkogkas et al., 2013; Santini et al., 2013), opening up new possibilities in terms of potential therapeutics. Much of the focus has been on the postsynaptic side of the synapse, the predominant site of plasticity and learning. Recent evidence indicates that regulated protein synthesis in the presynaptic compartment is also important for synapse formation (Taylor et al., 2013) and axon arborization (Hörnberg and Holt, 2013; Hörnberg et al., 2013; Kalous et al., 2013), raising the question of whether defects in axonal protein synthesis contribute to the miswiring aspects of neurodevelopmental disorders. Dysregulated protein synthesis may also underlie a broad range of neurodegenerative disorders (Fallini et al., 2012; Liu-Yesucevitz et al., 2011) consistent with axonal protein synthesis being required for axon maintenance (Hillefors et al., 2007; Yoon et al., 2012). Indeed, the first "effective" oral drug treatment that prevents neurodegeneration in a prion disease/Alzheimer's mouse model targets a kinase (PERK) that shuts down protein synthesis as part of the unfolded protein response (Moreno et al., 2013).

Summary

Recent years have witnessed a transformation in our appreciation of RNA function in dendrites/axons on the one hand and of neuronal compartments as spatially distinct signaling/processing units on the other. Here we have highlighted the convergence of these two areas and have sought to define some of the many interesting questions and challenges that lie ahead. As technical approaches become increasingly sensitive for unbiased profiling there is the promise of improved "understanding" of the qualitative concepts that govern the various active RNA species and formation and function of compartments as well as quantitative details on the stoichiometries of all of the players positioned within the morphological framework of the neuron and its remarkable dendritic and axonal arbor.

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