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Single molecule insights into mRNA dynamics in neurons

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Abstract

Targeting of mRNAs to neuronal dendrites and axons plays an integral role in intracellular signaling, development and synaptic plasticity. Single molecule imaging of mRNAs in neurons and brain tissue has led to enhanced understanding of mRNA dynamics. Here we discuss aspects of mRNA regulation as revealed by single molecule detection, which has led to quantitative analyses of mRNA diversity, localization, transport, and translation. These exciting new discoveries propel our understanding of the life of an mRNA in neurons and how its activity is regulated at the single molecule level.

Keywords

RNA localization; mRNA tracking; local translation; smFISH

RNA localization in neurons

Neuron morphology, a complex arborization of hundreds of branches up to millimeters in length, raises the biological enigma of how proteins can be targeted to their appropriate destinations. Neural activity-dependent modifications in the molecular composition of neurons and synapses underlie normal brain function and behavioral changes [1]. One mechanism to precisely target proteins is through the transport of mRNAs to distal regions in neuronal branches combined with local protein synthesis [2]. There is much evidence to suggest that mRNA localization and local translation in neurites is important for proper

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neuronal physiology [3–5]. For example, synthesis of new proteins is necessary for the induction of synaptic plasticity [6–9] (reviewed in [10]). Considerable effort has been made to understand how mRNAs are targeted to distal regions in neurons, what RNAs are present and under which conditions, and how translation is regulated (reviewed in [2,11]). As improved mRNA labeling methods and optical sensitivity have increased, so has our understanding of these questions. Techniques to visualize single mRNAs in fixed and live cells deliver the highest intracellular resolution of endogenous single mRNAs in non-neuronal cells (reviewed in [12]) and can now be applied to the field of neurobiology. The advantage of single molecule methods is that they provide absolute quantitative measurements of RNA expression. Single molecule imaging allows the measurement of discrete states rather than ensemble averages; therefore, it reveals subpopulations of molecules or transient states of dynamic processes. These properties of single molecule detection have provided important advantages for studying regulation of RNAs within all cells. The ability to identify single RNAs increases the confidence in expression abundance, whereas lower resolution techniques have a higher probability of false positives because the threshold of a specific signal is undefined. In live neurons, single molecule approaches have expanded our ability to explore the precise mechanism of RNA motility and localization [13,14]. Recent work highlighted here, utilizing single molecule fluorescence *in situ* hybridization (FISH) to endogenous mRNAs and live imaging of single mRNA in neurons has resulted in novel and at times even reformed insights into how mRNAs are localized in the brain [15–17]. For the sake of simplicity, technologies which are capable of yielding single molecule resolution are discussed as single molecule imaging without focusing on whether this sensitivity was achieved in each study. We further discuss insights into neuronal mRNA movements, targeting and translational regulation provided by single mRNA imaging, and what the future hopefully holds for the field.

Neuronal mRNA distribution is highly diverse

The elongated morphology of neurites implies that active transport plays a role in the delivery of RNAs to distal regions. Due to the high energy demands of active transport, RNA localization must be selective for those RNAs with critical local roles. The hierarchical and segregated structure of the hippocampus facilitates the differentiation of somatic and neurite layers, offering a platform to address this hypothesis via the quantification of RNA in different layers. Indeed, early demonstration of *in situ* hybridization in hippocampal slices distinguished mRNAs that were present in dendrites from those which were selectively excluded [18–20]. Moreover, contrasting patterns of mRNA localization in different hippocampal subfields strongly suggested that not only are neurons capable of sorting mRNAs, but that localization of a single species of mRNA could vary between neuronal subtypes and brain regions [18,21–24]. How the differential subcellular localization of a single mRNA species is accomplished remains unknown. Potentially, high-resolution RNA imaging methods can shed light on this important question. Using deep sequencing, more than 2500 different mRNAs have been identified in the synaptic neuropil of the hippocampus [25]. Moreover, *in vitro* single molecule imaging of the neuropil transcriptome by nanostring technique revealed that the relative mRNA abundances varied in excess of a thousand-fold [25]. The sensitivity of the assay demonstrated that many species of mRNAs

are present in widely varying amounts in the neuropil. To complement these and other unbiased techniques, single molecule detection of endogenous mRNAs has enabled researchers to probe the precise quantity and the exact location of each transcript within single neurons in culture (Figure 1). Further, single molecule labeling of endogenous RNA in brain tissue is beginning to put *in vitro* measurements into their biological context [14,16,25–29] and can even quantify the co-expression of distinct mRNA species in a single neuron *in vivo* [28]. The presence of such a diverse population of mRNA in neurites underscores the significance of compartmentalized gene expression in neurons and indicates that a large repertoire of proteins may be necessary to achieve neuronal function within specific compartments.

Mechanisms of mRNA localization in neurons

Much attention has been dedicated to understanding how mRNAs and RNA binding proteins (RBPs) interact to achieve motor based mRNA transport in neurons. Directed RNA transport requires binding of cis-acting RNA elements and RBPs, which mediate interactions with molecular motors that transport along microtubules (reviewed in [30]). Actively transported mRNAs typically contain specific cis-acting sequences or targeting elements that confer subcellular localization [31–35], (reviewed in [36]). These elements, typically located within the 3'-untranslated region (UTR) of the mRNA, interact with RBPs and other proteins to form mRNP (messenger ribonucleoprotein) complexes that are transported along microtubules by kinesin or dynein (reviewed in [37]) (Figure 2). Significant effort has been dedicated to characterizing localization elements and minimal functional elements and to understanding how they contribute to neuronal mRNA transport and localization [31–35]. Single molecule FISH can be used to measure the relative contribution of targeting elements or RBPs on localization. For instance, quantification of the localization of reporter mRNAs with alternate spinophilin dendritic targeting elements (DTE) directly demonstrated how the presence of the DTE sequence in the mRNA affects absolute mRNA counts in dendrites [38]. In lieu of direct visualization of mRNAs, fluorescently-tagged RBPs can function as a proxy to reveal dynamics of mRNA transport in neurites [39–42]. Collectively, RBP imaging studies have revealed that while most mRNPs were stationary, a subpopulation exhibited persistent and oscillatory bi-directional transport in neurites punctuated by pauses in motion. Loss or mutation of RBPs, combined with live tracking, has also been informative for the contribution of individual RBPs to active transport. Amyotrophic lateral sclerosis (ALS) associated mutations in the TDP-43 altered the transport capability of the mRNP and reduced the net displacements of the mRNP in motor axons [42]. Similarly, a reduced mRNP motility was observed in *Drosophila* neurons lacking Fragile X Mental Retardation Protein (FMRP) compared to wildtype [43]. Consistent with the importance of properly regulated intracellular RBP-mRNA stoichiometry [44], overexpressing *Drosophila* FMRP also resulted in decreased mRNP movement, presumably due to excessive recruitment of motors to mRNPs, which can inhibit active transport [43]. These results suggest the importance of proper mRNA transport in neuronal function, because mutations in both TDP-43 and FMRP are known links to cognitive disorders.

Single mRNA movement in neurites

Direct visualization of individual mRNAs in live neurons has yielded an enhanced understanding of mRNA transport dynamics. By tracking the movement of single mRNAs, changes in velocity or direction during motor based transport can indicate regulation of motor binding and activity [45,46]. Measurement of mRNA diffusion rates can correlate mRNA movement with the cellular milieu. In neurons, high resolution imaging of active transport and localization of mRNAs can be accomplished by microinjecting labeled mRNAs [31,47,48] or by using the MS2-GFP system [13,49–57] (see Text Box 1). Observing the movements of mRNA in neurons lacking specific RBPs has yielded information on how RBPs regulate mRNA transport behavior. Live imaging of MS2 (Box 1) reporter mRNA demonstrated that Huntingtin (Htt), huntingtin-associated protein 1 (HAP1), KIF5A kinesin, and dynein colocalized with β -actin mRNA during dendritic transport, and knock-down of these proteins reduced the transport of β -actin mRNA [57]. This study directly demonstrated that active mRNA trafficking in neurons was mediated by members of the kinesin and dynein motor families. Tracking reporter RNAs using the MS2 labeling system in neurons lacking FMRP revealed that FMRP can function as an adaptor for the kinesin light chain [50] and deliver CamKII α mRNA to dendritic spines in response to synaptic stimulation [52].

The aforementioned mRNA tracking studies have relied on transfection or injection of exogenous RNA. However an exogenous reporter RNA may not recapitulate the full repertoire of behavior of endogenous RNA for multiple reasons. Reporter RNAs are usually over-expressed and may lack some of the cis-regulatory elements and transacting RBPs because they do not undergo proper splicing and processing in the nucleus. In order to label endogenous mRNA, a recent study used knock-in and transgenic techniques and extended the utility of MS2-GFP system in live mice [14,26]. Tracking labeled, endogenous single β -actin mRNA revealed that RNA movements in neuronal dendrites are distinct from those in fibroblasts. Diffusion of β -actin mRNA in neurons ($D \approx 0.004 \mu\text{m}^2$) was ~ 24 times slower than in fibroblasts suggesting a more confined environment. While mRNA movements in fibroblasts were dominated by diffusive motion, the majority of the endogenous β -actin mRNA was stationary in neurons consistent with the results obtained from using exogenous reporter RNA [49,52]. If travel in one direction for more than $1.5 \mu\text{m}$ is classified as directed motion, about 10% of mRNPs showed bi-directional motion with a mean speed of $1.3 \mu\text{m/s}$. The frequency ratio of anterograde to retrograde movement was approximately 1.3 suggesting that a bias to the anterograde direction mediates the constitutive delivery of β -actin mRNAs to distal dendrites. The ability to measure the dynamics of single mRNAs revealed the precise kinetics for sub-populations of mRNAs.

Finding the destination

Observation of the oscillatory behavior of a fraction of actively transported mRNAs in neurites suggests a scanning behavior near areas that may require local translation such as synapses. Given that synaptic transmission and modification of the efficacy of transmission strength can be specific to single dendritic spines [58], it is likely that mRNA localization may also be specific for individual spines. An attractive model for mRNA targeting to

stimulated synapses is that mRNAs transport stochastically along neurites and are captured or stalled at sites of stimulation [32,59] (Figure 2). This model conveniently circumvents a mechanism of pre-determining a destination for a traveling mRNA. Stimulated synapses may express a synaptic tag (reviewed in [60]), which leads to mRNA capture, thus targeting protein synthesis to locations requiring modifications [32]. Single Arc and β -actin mRNAs have been shown to aggregate or cluster in certain regions, suggesting that specific areas in dendrites may be more prone to induce mRNA pausing or stalling [13,14,61]. Live observations of transfected Arc reporter mRNA revealed that actively transported mRNAs often reduce velocity before coming to a stop [13] and single Arc mRNAs are stably positioned at the base of spines [51]. Furthermore, in some cases, actively transported mRNAs may "overshoot" a spine, reverse direction and return before arresting motility [13]. Observations of these behaviors may contribute insight into how an mRNP is instructed to stop or travel to a synapse. *In silico* measurements show that local cues such as metabolic gradients which cause motor detachment from a randomly searching mRNA cargo can efficiently deliver multiple cargos to their targets [62,63]. These studies suggest that by using a motile fraction of randomly searching mRNAs and by altering their motility in response to local cues, docking at specific synapses can likely be quickly and efficiently achieved.

Exploring molecular candidates responsible for mRNA docking at synapses is starting to illuminate mechanisms of targeting mRNAs. For example, FMRP, an RBP with roles in regulating mRNA transport and translation, has been shown to play an important role in translocating CaMKII α mRNA to dendritic spines after stimulation of metabotropic glutamate receptors (mGluRs) [52,64]. The RBP FUS/TLS (Fused in Sarcoma/Translocated in Liposarcoma) also moves to dendritic spines in response to mGluR stimulation [65]. In addition, molecular motors themselves may participate in delivering or docking mRNAs to synapses. The actin based motor Myosin Va has a role in mRNP accumulation at actin rich dendritic spines [66]. A number of RBPs can associate with actin filaments by interacting with Myosin Va, leading to a mechanism of mRNA and RBP docking at dendritic spines [65,67]. Nevertheless, there are still many questions: how mRNAs are induced to pause at specified locations and how transport is regulated to achieve precise positioning. By combining single mRNA imaging with perturbation of RBPs or other molecules that play a role in mRNA docking, mechanisms of specific mRNA targeting to synapses can be revealed.

Seeing the tree from the forest – changed perceptions

Prior to single mRNA detection, visualization of mRNAs lacked sufficient sensitivity and spatial resolution, making the quantification of mRNAs and their exact positions difficult. With high resolution techniques some discrepancies have emerged on the abundance of certain localized mRNAs in neurons. Due to the highly diverse and abundant nature of localized neuronal mRNA, early reports proposed that numerous localized mRNAs traveled together as transport granules. This conclusion addressed how multiple mRNAs could be induced to translate in unison and contribute a pool of synaptic or plasticity related proteins, as well as increase the efficiency of mRNA transport. Microinjected CaMKII α and Septin7 RNAs, which share similar dendritic targeting elements (DTE), may colocalize around 10%

of the time in the same particles in dendrites [31]. Single molecule imaging of endogenous β -actin mRNA in neurons revealed continuous assembly and disassembly of large mRNP complexes containing multiple copies of β -actin mRNAs [13].

However, some single molecule studies in cultured neurons demonstrated that different mRNA species do not colocalize to a great extent [15,68]. In brain tissue, Arc and CaMKII α mRNAs, which share A2 DTEs, were present in Dentate Gyrus dendrites following stimulation, however in distinct populations of particles [16]. Whether mRNAs are single or clustered may depend on the transient masking effect of mRNAs in granules where they are not accessible by FISH probes, particularly in unstimulated neurons [61]. In addition, there is an inherently low probability of finding granules that contain two specific mRNA species chosen for each FISH analysis while there can be many other mRNA species that are not labeled. Therefore aforementioned studies may not necessarily be in opposition as they all observe a low amount of coalescing mRNAs ranging from 2% to 10%.

Since high-resolution detection of mRNA can determine the distribution of compartmentalized mRNA, it can also determine certain transcripts that are absent. Recent studies utilizing FISH to detect endogenous mRNA have led to some alternative interpretations of dendritically localized mRNA. For example, the nascent transcripts encoding brain-derived neurotrophic factor (BDNF) undergo alternative splicing, which results in two isoforms with different 3' UTR lengths (short and long). Previously, it had been shown that the long 3'UTR isoform of BDNF mRNA was capable of trafficking to distal dendrites [69]. However, high resolution FISH revealed only sparse amounts of BDNF mRNA in the soma relative to other neuronal transcripts, and no detection of the long isoform in distal dendrites [70]. In addition, FISH of neurotransmitter receptor mRNA provided new insight into the localization and regulation of the post-synaptic response to the neurotransmitter glutamate. AMPA (α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, a type of glutamate receptor, may be composed of various combinations of subunits (GluA 1–4) and inclusion of the GluA2 subunit selectively diminishes the receptor's permeability to extracellular calcium. Branched DNA labeling of endogenous mRNA showed that the GluA2 subunit mRNA of the AMPA receptor was not present in dendrites but instead was largely restricted to the soma [17]. In contrast, earlier studies reported dendritic localization of AMPA receptor subunit mRNAs (GluA1–4) and that subsequent synthesis of AMPA receptors could be modulated locally in an activity-dependent manner [71–75]. These results demonstrated that the enhanced sensitivity of single molecule assays may alter our assumptions concerning mRNA abundance and location. Due to the central role glutamate receptors play in synaptic transmission, it is likely that neurons have developed multiple and redundant ways to modulate expression during periods of plasticity. Moreover, differential neuronal signaling properties could lead to different AMPA receptor mRNA expression and localization profiles in various neuronal subtypes and brain regions [24]. While improvements in detection of RNA in neurons will inevitably introduce some discrepancies with previously published reports, the findings will eventually enhance our understanding of local protein synthesis in neurons.

Localization is only half the battle: regulating local translation of mRNAs in processes

While the localization of mRNAs into dendrites implies the occurrence of local translation, visual measurements of active local translation have been scarcer and have yielded lower resolution measurements than mRNA imaging in neurons [75–80]. It would be illuminating to complement the visualization of mRNA localization with measurements of translational status and quantitative translational output of mRNAs.

Due to the length of neurites, the delivery of mRNAs may be slow, and certain mRNAs are thought to translate locally in response to a synaptic cue; therefore, it is likely that constitutively localized mRNAs are largely in a translationally repressed state in dendrites [61,81]. By regulating both location and translational status of mRNAs, neurons can orchestrate local gene expression with high spatial fidelity.

Many RBPs prevent translation while transporting mRNAs. Moreover, higher order mRNP complexes in neurons such as RNA granules, which are composed of many RBPs and ribosomes, are integral in maintaining translational repression of the mRNAs they contain. The ability to detect β -actin mRNA with single molecule FISH was used to report on the presence of single mRNAs in repressive granules thereby indicating their translational status [61]. Single molecule detection quantitatively demonstrated that β -actin mRNAs in repressive structures were released during synaptic stimulation. This also showed that mRNA detection in neurons may be subject to obscuring structures, necessitating more aggressive detection methods [61] [82].

Other single molecule assays for translation have utilized imaging of a fluorescent protein reporter to assess rates of translation in neurons. Using the fast folding, bright, and easily bleached fluorescent protein Venus revealed that stimulation of metabotropic glutamate receptors or inhibition of action potentials increased and decreased, respectively the translation of Venus-ARC and Venus-FMRP reporters within minutes [48]. Translation of Venus-PSD95 synaptic protein also increased in response to metabotropic glutamate stimulation [83]. Neurons lacking the translational regulator FMRP lost the ability to regulate translation at steady state as well as in response to the stimulation [83]. In contrast to other methods that require minutes to hours to detect an appreciable amount or change in protein synthesis [76,77,79,80,84,85], the ability to count single events with this method greatly enhanced the time resolution of monitoring changes in translation.

Beyond the messenger

While it is now possible to detect and image messenger RNA at the single molecule level, the advent of imaging technologies in conjunction with RNA labeling techniques also allows high resolution detection of non-coding RNAs. Quantitative amplification of fluorescent signal using branched oligo FISH labeling can facilitate detection of sequences as short as 20 nucleotides in length. In the study of single molecule GluA2 mRNA in dendrites, single transcripts of miR-124 were observed along dendrites, offering novel *in situ* insight into the regulation of local translation via microRNAs [17]. Similarly, Ube3a mRNA, which

produces a seemingly non-functional protein, localizes to dendrites and plays a role in regulating the availability of miRNA-134, is localized to dendrites [82]. Branched DNA FISH also allowed the detection of single circular forms of RNAs, which are highly enriched in neural tissue, by targeting probes to the head-to-tail junction [27]. This facilitated the observation that the expression of circular forms of certain mRNAs can be modified during development and by activity. The use of high resolution FISH techniques to address the precise abundance and localization of mRNAs, non-coding RNAs, and even mRNA transcription dynamics (Text Box 2) will describe the multifaceted regulatory mechanisms of localized gene expression.

Concluding remarks

mRNA transport and local translation have been increasingly recognized as important mechanisms of neural plasticity and development. Recent studies using single molecule imaging of mRNA have shed light on detailed aspects of mRNA dynamics with unprecedented spatio-temporal resolution. Digital detection of individual molecules has enabled quantitative measurements even for a trace amount of RNA inside single neurons. Recent advances in RNA labeling in situ has allowed the visualization of miRNAs, non-coding RNAs and circular RNAs, expanding the repertoire of visualization beyond messages.

Observations of highly heterogeneous distribution and movement of mRNPs have revealed complex interactions of mRNA, RBP, and motor proteins for the regulation of local gene expression. However, the mechanisms of targeting mRNAs to specific sites and regulation of local translation are still largely unknown (See Text Box 3). It is important to continue to advance single molecule imaging technologies for visualizing RNA-protein interactions as well as local translation processes in real time. In addition, further work is needed to investigate RNA dynamics in the intact neural network in live animals to understand the physiological consequences of normal and disrupted RNA regulation.

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References

1. Hanus C, Schuman EM. Proteostasis in complex dendrites. *Nat Rev Neurosci.* 2013; 14:638–648. [PubMed: 23900412]
2. Holt CE, Schuman EM. The Central Dogma Decentralized: New Perspectives on RNA Function and Local Translation in Neurons. *Neuron.* 2013; 80:648–657. [PubMed: 24183017]
3. Buffington SA, et al. Translational control in synaptic plasticity and cognitive dysfunction. *Annu Rev Neurosci.* 2014; 37:17–38. [PubMed: 25032491]
4. Costa-Mattioli M, et al. Translational control of long-lasting synaptic plasticity and memory. *Neuron.* 2009; 61:10–26. [PubMed: 19146809]
5. Liu-Yesucevitz L, et al. Local RNA translation at the synapse and in disease. *J Neurosci.* 2011; 31:16086–16093. [PubMed: 22072660]

6. Casadio A, et al. A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. *Cell*. 1999; 99:221–237. [PubMed: 10535740]
7. Huber KM, et al. Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science*. 2000; 288:1254–1257. [PubMed: 10818003]
8. Kang H, Schuman EM. A requirement for local protein synthesis in neurotrophin-induced hippocampal synaptic plasticity. *Science*. 1996; 273:1402–1406. [PubMed: 8703078]
9. Martin KC, et al. Synapse-specific, long-term facilitation of aplysia sensory to motor synapses: a function for local protein synthesis in memory storage. *Cell*. 1997; 91:927–938. [PubMed: 9428516]
10. Martin KC, et al. Local protein synthesis and its role in synapse-specific plasticity. *Curr Opin Neurobiol*. 2000; 10:587–592. [PubMed: 11084321]
11. Hutten S, et al. Unmasking the messenger. *RNA Biol*. 2014:e32091.
12. Buxbaum AR, et al. In the right place at the right time: visualizing and understanding mRNA localization. *Nat Rev Mol Cell Biol*. 2015; 16:95–109. [PubMed: 25549890]
13. Dynes JL, Steward O. Dynamics of bidirectional transport of Arc mRNA in neuronal dendrites. *J Comp Neurol*. 2007; 500:433–447. [PubMed: 17120280]
14. Park HY, et al. Visualization of dynamics of single endogenous mRNA labeled in live mouse. *Science*. 2014; 343:422–424. [PubMed: 24458643]
15. Batish M, et al. Neuronal mRNAs travel singly into dendrites. *P Natl Acad Sci USA*. 2012; 109:4645–4650.
16. Farris S, et al. Selective localization of arc mRNA in dendrites involves activity- and translation-dependent mRNA degradation. *J Neurosci*. 2014; 34:4481–4493. [PubMed: 24671994]
17. Ho VM, et al. Gl uA2 mRNA distribution and regulation by miR-124 in hippocampal neurons. *Mol Cell Neurosci*. 2014; 61:1–12. [PubMed: 24784359]
18. Garner CC, et al. Selective Localization of Messenger-Rna for Cytoskeletal Protein Map2 in Dendrites. *Nature*. 1988; 336:674–677. [PubMed: 3200318]
19. Burgin KE, et al. In situ hybridization histochemistry of Ca²⁺/calmodulin-dependent protein kinase in developing rat brain. *J Neurosci*. 1990; 10:1788–1798. [PubMed: 2162385]
20. Kleiman R, et al. Differential subcellular localization of particular mRNAs in hippocampal neurons in culture. *Neuron*. 1990; 5:821–830. [PubMed: 2148488]
21. Watson JB, et al. G-Protein Gamma-7 Subunit Is Selectively Expressed in Medium-Sized Neurons and Dendrites of the Rat Neostriatum. *J Neurosci Res*. 1994; 39:108–116. [PubMed: 7807587]
22. Paradies MA, Steward O. Multiple subcellular mRNA distribution patterns in neurons: a nonisotopic in situ hybridization analysis. *J Neurobiol*. 1997; 33:473–493. [PubMed: 9322162]
23. Berry FB, Brown IR. CaM I mRNA is localized to apical dendrites during postnatal development of neurons in the rat brain. *J Neurosci Res*. 1996; 43:565–575. [PubMed: 8833091]
24. Cox DJ, Racca C. Differential dendritic targeting of AMPA receptor subunit mRNAs in adult rat hippocampal principal neurons and interneurons. *J Comp Neurol*. 2013; 521:1954–2007. [PubMed: 23296627]
25. Cajigas IJ, et al. The Local Transcriptome in the Synaptic Neuropil Revealed by Deep Sequencing and High-Resolution Imaging. *Neuron*. 2012; 74:453–466. [PubMed: 22578497]
26. Lionnet T, et al. A transgenic mouse for in vivo detection of endogenous labeled mRNA. *Nat Methods*. 2011; 8:165–170. [PubMed: 21240280]
27. You X, et al. Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. *Nat Neurosci*. 2015; 18:603–610. [PubMed: 25714049]
28. Ghamari-Langroudi M, et al. G-protein-independent coupling of MC4R to Kir7.1 in hypothalamic neurons. *Nature*. 2015
29. Zhang HY, et al. Species differences in cannabinoid receptor 2 and receptor responses to cocaine self-administration in mice and rats. *Neuropsychopharmacology*. 2015; 40:1037–1051. [PubMed: 25374096]
30. Czaplinski K. Understanding mRNA trafficking: Are we there yet? *Semin Cell Dev Biol*. 2014; 32:63–70. [PubMed: 24769369]

31. Tubing F, et al. Dendritically Localized Transcripts Are Sorted into Distinct Ribonucleoprotein Particles That Display Fast Directional Motility along Dendrites of Hippocampal Neurons. *J Neurosci.* 2010; 30:4160–4170. [PubMed: 20237286]
32. Doyle M, Kiebler MA. Mechanisms of dendritic mRNA transport and its role in synaptic tagging. *EMBO J.* 2011; 30:3540–3552. [PubMed: 21878995]
33. Kobayashi H, et al. Identification of a cis-acting element required for dendritic targeting of activity-regulated cytoskeleton-associated protein mRNA. *Eur J Neurosci.* 2005; 22:2977–2984. [PubMed: 16367764]
34. Subramanian M, et al. G-quadruplex RNA structure as a signal for neurite mRNA targeting. *Embo Reports.* 2011; 12:697–704. [PubMed: 21566646]
35. Meer EJ, et al. Identification of a cis-acting element that localizes mRNA to synapses. *P Natl Acad Sci USA.* 2012; 109:4639–4644.
36. Andreassi C, Riccio A. To localize or not to localize: mRNA fate is in 3'UTR ends. *Trends Cell Biol.* 2009; 19:465–474. [PubMed: 19716303]
37. Xing L, Bassell GJ. mRNA Localization: An Orchestration of Assembly, Traffic and Synthesis. *Traffic.* 2013; 14:2–14. [PubMed: 22913533]
38. Patel VL, et al. Spatial arrangement of an RNA zipcode identifies mRNAs under post-transcriptional control. *Genes Dev.* 2012; 26:43–53. [PubMed: 22215810]
39. Kohrmann M, et al. Microtubule-dependent recruitment of Staufen-Green fluorescent protein into large RNA-containing granules and subsequent dendritic transport in living hippocampal neurons. *Mol Biol Cell.* 1999; 10:2945–2953. [PubMed: 10473638]
40. Zhang HL, et al. Neurotrophin-induced transport of a beta-actin mRNP complex increases beta-actin levels and stimulates growth cone motility. *Neuron.* 2001; 31:261–275. [PubMed: 11502257]
41. Tiruchinapalli DM, et al. Activity-dependent trafficking and dynamic localization of zipcode binding protein 1 and beta-actin mRNA in dendrites and spines of hippocampal neurons. *J Neurosci.* 2003; 23:3251–3261. [PubMed: 12716932]
42. Alami NH, et al. Axonal transport of TDP-43 mRNA granules is impaired by ALS-causing mutations. *Neuron.* 2014; 81:536–543. [PubMed: 24507191]
43. Estes PS, et al. Fragile X protein controls the efficacy of mRNA transport in Drosophila neurons. *Mol Cell Neurosci.* 2008; 39:170–179. [PubMed: 18655836]
44. Donnelly CJ, et al. Limited availability of ZBP1 restricts axonal mRNA localization and nerve regeneration capacity. *EMBO J.* 2011; 30:4665–4677. [PubMed: 21964071]
45. Bullock SL. Translocation of mRNAs by molecular motors: think complex? *Semin Cell Dev Biol.* 2007; 18:194–201. [PubMed: 17324596]
46. Park HY, et al. Single mRNA tracking in live cells. *Methods Enzymol.* 2010; 472:387–406. [PubMed: 20580973]
47. Gao Y, et al. Multiplexed dendritic targeting of alpha calcium calmodulin-dependent protein kinase II, neurogranin, and activity-regulated cytoskeleton-associated protein RNAs by the A2 pathway. *Mol Biol Cell.* 2008; 19:2311–2327. [PubMed: 18305102]
48. Tatavarty V, et al. Single-molecule imaging of translational output from individual RNA granules in neurons. *Mol Biol Cell.* 2012; 23:918–929. [PubMed: 22219377]
49. Rook MS, et al. CaMKIIalpha 3' untranslated region-directed mRNA translocation in living neurons: visualization by GFP linkage. *J Neurosci.* 2000; 20:6385–6393. [PubMed: 10964944]
50. Dictenberg JB, et al. A direct role for FMRP in activity-dependent dendritic mRNA transport links filopodial-spine morphogenesis to fragile X syndrome. *Dev Cell.* 2008; 14:926–939. [PubMed: 18539120]
51. Dynes JL, Steward O. Arc mRNA docks precisely at the base of individual dendritic spines indicating the existence of a specialized microdomain for synapse-specific mRNA translation. *J Comp Neurol.* 2012; 520:3105–3119. [PubMed: 22350812]
52. Kao DI, et al. Altered mRNA transport, docking, and protein translation in neurons lacking fragile X mental retardation protein. *P Natl Acad Sci USA.* 2010; 107:15601–15606.
53. Jo A, Kim HK. Up-regulation of dendritic Kv4.2 mRNA by activation of the NMDA receptor. *Neurosci Lett.* 2011; 496:129–134. [PubMed: 21511008]

54. Oh JY, et al. Apolipoprotein E mRNA is transported to dendrites and may have a role in synaptic structural plasticity. *J Neurochem.* 2010; 114:685–696. [PubMed: 20456011]
55. Bi J, et al. Axonal mRNA transport and localized translational regulation of kappa-opioid receptor in primary neurons of dorsal root ganglia. *P Natl Acad Sci USA.* 2006; 103:19919–19924.
56. Bi J, et al. Copb1-facilitated axonal transport and translation of kappa opioid-receptor mRNA. *P Natl Acad Sci USA.* 2007; 104:13810–13815.
57. Ma B, et al. Huntingtin mediates dendritic transport of beta-actin mRNA in rat neurons. *Sci Rep.* 2011; 1:140. [PubMed: 22355657]
58. Matsuzaki M, et al. Structural basis of long-term potentiation in single dendritic spines. *Nature.* 2004; 429:761–766. [PubMed: 15190253]
59. Steward O, et al. Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. *Neuron.* 1998; 21:741–751. [PubMed: 9808461]
60. Frey S, Frey JU. 'Synaptic tagging' and 'cross-tagging' and related associative reinforcement processes of functional plasticity as the cellular basis for memory formation. *Prog Brain Res.* 2008; 169:117–143. [PubMed: 18394471]
61. Buxbaum AR, et al. Single beta-actin mRNA detection in neurons reveals a mechanism for regulating its translatability. *Science.* 2014; 343:419–422. [PubMed: 24458642]
62. Newby JM, Bressloff PC. Quasi-steady state reduction of molecular motor-based models of directed intermittent search. *Bull Math Biol.* 2010; 72:1840–1866. [PubMed: 20169417]
63. Newby J, Bressloff PC. Local synaptic signaling enhances the stochastic transport of motor-driven cargo in neurons. *Phys Biol.* 2010; 7:036004. [PubMed: 20733246]
64. Ferrari F, et al. The fragile X mental retardation protein-RNP granules show an mGluR-dependent localization in the post-synaptic spines. *Molecular and Cellular Neuroscience.* 2007; 34:343–354. [PubMed: 17254795]
65. Fujii R, et al. The RNA binding protein TLS is translocated to dendritic spines by mGluR5 activation and regulates spine morphology. *Current Biology.* 2005; 15:587–593. [PubMed: 15797031]
66. Yoshimura A, et al. Myosin-Va facilitates the accumulation of mRNA/protein complex in dendritic spines. *Curr Biol.* 2006; 16:2345–2351. [PubMed: 17141617]
67. Nalavadi VC, et al. Regulation of zipcode binding protein 1 transport dynamics in axons by myosin Va. *J Neurosci.* 2012; 32:15133–15141. [PubMed: 23100434]
68. Mikl M, et al. Independent localization of MAP2, CaMKIIalpha and beta-actin RNAs in low copy numbers. *EMBO Rep.* 2011; 12:1077–1084. [PubMed: 21869818]
69. An JJ, et al. Distinct role of long 3' UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. *Cell.* 2008; 134:175–187. [PubMed: 18614020]
70. Will TJ, et al. Deep sequencing and high-resolution imaging reveal compartment-specific localization of Bdnf mRNA in hippocampal neurons. *Sci Signal.* 2013; 6:rs16. [PubMed: 24345682]
71. Miyashiro K, et al. On the nature and differential distribution of mRNAs in hippocampal neurites: implications for neuronal functioning. *P Natl Acad Sci USA.* 1994; 91:10800–10804.
72. Kacharmina JE, et al. Stimulation of glutamate receptor protein synthesis and membrane insertion within isolated neuronal dendrites. *P Natl Acad Sci USA.* 2000; 97:11545–11550.
73. Ju W, et al. Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nat Neurosci.* 2004; 7:244–253. [PubMed: 14770185]
74. Grooms SY, et al. Activity bidirectionally regulates AMPA receptor mRNA abundance in dendrites of hippocampal neurons. *J Neurosci.* 2006; 26:8339–8351. [PubMed: 16899729]
75. Kim TK, et al. Dendritic glutamate receptor mRNAs show contingent local hotspot-dependent translational dynamics. *Cell Rep.* 2013; 5:114–125. [PubMed: 24075992]
76. Aakalu G, et al. Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron.* 2001; 30:489–502. [PubMed: 11395009]
77. Raab-Graham KF, et al. Activity- and mTOR-dependent suppression of Kv1.1 channel mRNA translation in dendrites. *Science.* 2006; 314:144–148. [PubMed: 17023663]

78. Sutton MA, et al. Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell*. 2006; 125:785–799. [PubMed: 16713568]
79. Swanger SA, et al. Dendritic GluN2A synthesis mediates activity-induced NMDA receptor insertion. *J Neurosci*. 2013; 33:8898–8908. [PubMed: 23678131]
80. Wang DO, et al. Synapse- and stimulus-specific local translation during long-term neuronal plasticity. *Science*. 2009; 324:1536–1540. [PubMed: 19443737]
81. Graber TE, et al. Reactivation of stalled polyribosomes in synaptic plasticity. *Proc Natl Acad Sci U S A*. 2013; 110:16205–16210. [PubMed: 24043809]
82. Valluy J, et al. A coding-independent function of an alternative Ube3a transcript during neuronal development. *Nat Neurosci*. 2015; 18:666–673. [PubMed: 25867122]
83. Ifrim MF, et al. Single-Molecule Imaging of PSD-95 mRNA Translation in Dendrites and Its Dysregulation in a Mouse Model of Fragile X Syndrome. *J Neurosci*. 2015; 35:7116–7130. [PubMed: 25948262]
84. Smith WB, et al. Dopaminergic stimulation of local protein synthesis enhances surface expression of GluR1 and synaptic transmission in hippocampal neurons. *Neuron*. 2005; 45:765–779. [PubMed: 15748851]
85. Dieterich DC, et al. In situ visualization and dynamics of newly synthesized proteins in rat hippocampal neurons. *Nat Neurosci*. 2010; 13:897–905. [PubMed: 20543841]
86. Femino AM, et al. Visualization of single RNA transcripts in situ. *Science*. 1998; 280:585–590. [PubMed: 9554849]
87. Kwon S. Single-molecule fluorescence in situ hybridization: quantitative imaging of single RNA molecules. *BMB Rep*. 2013; 46:65–72. [PubMed: 23433107]
88. Raj A, et al. Imaging individual mRNA molecules using multiple singly labeled probes. *Nat Methods*. 2008; 5:877–879. [PubMed: 18806792]
89. Sinnamon JR, Czaplinski K. RNA detection in situ with FISH-STICs. *RNA*. 2014; 20:260–266. [PubMed: 24345395]
90. Bertrand E, et al. Localization of ASH1 mRNA particles in living yeast. *Mol Cell*. 1998; 2:437–445. [PubMed: 9809065]
91. Chao JA, et al. Structural basis for the coevolution of a viral RNA-protein complex. *Nat Struct Mol Biol*. 2008; 15:103–105. [PubMed: 18066080]
92. Larson DR, et al. Real-time observation of transcription initiation and elongation on an endogenous yeast gene. *Science*. 2011; 332:475–478. [PubMed: 21512033]
93. Hocine S, et al. Single-molecule analysis of gene expression using two-color RNA labeling in live yeast. *Nat Methods*. 2013; 10:119–121. [PubMed: 23263691]
94. Zenklusen D, et al. Single-RNA counting reveals alternative modes of gene expression in yeast. *Nat Struct Mol Biol*. 2008; 15:1263–1271. [PubMed: 19011635]
95. Darzacq X, et al. Imaging transcription in living cells. *Annu Rev Biophys*. 2009; 38:173–196. [PubMed: 19416065]
96. Guzowski JF, et al. Environment-specific expression of the immediate-early gene *Arc* in hippocampal neuronal ensembles. *Nat Neurosci*. 1999; 2:1120–1124. [PubMed: 10570490]
97. Guzowski JF, et al. Recent behavioral history modifies coupling between cell activity and *Arc* gene transcription in hippocampal CA1 neurons. *P Natl Acad Sci USA*. 2006; 103:1077–1082.
98. Perry RB, Fainzilber M. Local Translation in Neuronal Processes-In Vivo Tests of a "Heretical Hypothesis". *Dev Neurobiol*. 2014; 74:210–217. [PubMed: 23929725]

Highlights

- mRNA localization into neuronal processes and localized translation is an efficient mechanism of local protein delivery in neurons due to their long processes and synaptic function.
- Single mRNA studies in neurons illustrate the diverse and stochastic nature of mRNA trafficking into neuronal processes.
- Single molecule imaging of endogenous mRNA has illuminated an altered or unexpected understanding of mRNA dynamics.

Text Box 1**Imaging single mRNAs in neurons****Single molecule FISH**

Since the first demonstration of single molecule FISH [86], variations of FISH techniques have been used to detect individual transcripts with high accuracy (reviewed in [87]). Originally, five 50 nucleotide-long amino-modified oligodeoxynucleotides (ODNs), each of which is directly labeled with five fluorescent dyes, were used for FISH in neurons [50]. This method enables highly sensitive detection of single probes bearing five dyes as well as single mRNAs bearing up to 25 dyes [86]. The ability to count single fluorescent probes confirmed single molecule detection. An analogous single molecule FISH method was developed using 48 or more of 20 nt-long ODNs labeled with a single fluorophore [88]. This method enabled a cost-effective, high throughput synthesis of FISH probes, which contributed to the wide use of the technique. In addition, amplification of fluorescent signal on a shorter target can be achieved using dendrimers [25] or sequential tethering of ODNs [89], where the complementary oligo is further bound by non-targeting fluorescent sequences. The use of shorter target sequences may increase false positive or negative signal, however the fluorescence is significantly brighter due to the serial amplification of fluorescent dyes at the RNA.

Microinjection of labeled mRNA

Localization of mRNA in live neurons can be visualized by using microinjection of fluorescently labeled mRNAs [31,47]. Fluorescent RNAs are *in vitro* transcribed in the presence of UTPs labeled with a fluorescent dye. Labeled RNAs are introduced into live neurons using an injection needle with a pressure injection system. Although it is tedious and less efficient, microinjection has been an effective method to track individual mRNP particles.

Tagging RNA with GFP

Genetically encoded RNA reporters utilize highly specific binding between an RBP and its target RNA sequence. The MS2-GFP system, in which MS2 bacteriophage capsid protein fused with GFP (MCP-GFP) is co-expressed with an RNA tagged with the MS2 binding site (MBS), has been widely used to track single mRNAs (reviewed in [46]). In order to increase the signal-to-background ratio, a reporter RNA is tagged with 24 repeats of MBS stem-loops, each of which binds a dimer of MCP [90]. More recently, RNA-protein pairs derived from other bacteriophages such as λ and PP7 [91–93] have been developed for multi-color imaging of mRNAs in live cells (reviewed in [12]).

Text Box 2**Imaging Transcription Dynamics**

Advances in single molecule mRNA imaging have led to revelations of how mRNAs move and translate in neuronal processes; however, the technology also lends itself to a single molecule resolution of mRNA synthesis dynamics. FISH or live imaging of endogenous mRNAs combined with normalizing the total intensity of mRNAs present at sites of transcription to the intensity of single mRNAs can result in valuable information on transcription rates and kinetics [92,94,95]. For example, depolarization of hippocampal slices to stimulate neurons increases the number of nascent β -actin mRNAs [14]. Arc mRNA levels are low in steady state conditions, however environmental and electrical stimulations robustly activate Arc transcription and dendritic localization into neuronal dendrites [16,59]. To quantify mRNA synthesis in relation to a stimulus, FISH labelling of Arc transcription sites in brain slices is used as a measure of gene expression activation during behavior [96,97]. Quantitative assessment of the transcriptional dynamics of different mRNAs in hippocampal slices will illuminate how the transcriptional response to a stimulus varies among mRNAs and neuronal subtypes. These measurements will be critical for our understanding of how constitutively expressed and modular plasticity related genes differ in their regulatory mechanisms of expression during plasticity paradigms related to learning.

Text Box 3**Outstanding Questions**

- How is the differential expression of an mRNA species regulated in neuronal subtypes and brain regions? While our understanding of localization elements and their interaction with RBPs and motors describes how distinct mRNAs have contrasting localization, it is unexplained how the same mRNA exhibits divergent localization patterns depending on its context.
- What is the minimal cis-acting RNA sequence that determines RNA transport into dendrites and/or axons? Methodical identification of targeting and minimal targeting elements of neuronal genes will certainly reveal conserved sequences and axioms which target mRNAs into dendrites and axons.
- Many questions remain on the molecular composition of mRNPs and which proteins play roles in regulating the unique localization of individual mRNAs. These include: what are the adaptor proteins that recruit motor proteins to mRNP complexes in neurons? How specific are RBPs to one type of mRNA? Are there neuron specific RBPs or proteins that generate unique mRNA behavior in neurons such as docking at synapses or long distance transport? Enhancing our understanding of mRNP identity and function will lead to our understanding of how localization is determined.
- How are mRNPs recognized and captured at the activated dendritic regions or synapses? Identification of synaptic tags that lead to the capture of mRNAs and proteins following synaptic activity and to consolidation and memory formation will be transformative for our understanding of how local translation is directed to stimulated synapses.
- While dendritic mRNAs may dock at spines, how do mRNAs behave in axons? Although mRNAs have long been established to localize to dendrites as well as axons, information regarding the latter compartment has been subject to scrutiny due to claims of non-axonal mRNA contamination obfuscating legitimate axonally localized mRNAs [98]. Notwithstanding, there remains a great deal of opportunity to observe how mRNAs behave in axons using single molecule technologies. Optical techniques such as smFISH and live tracking may reveal that mRNAs, which have been previously characterized in dendrites, behave in different manners in axons. The unipolar microtubule orientation in axons may inhibit retrograde mRNA movements, which will be evident upon live observations of the same mRNA species in both types of neuronal processes.
- How do the transport kinetics of different mRNAs with different functions compare to those of well-characterized mRNAs such as β -actin and Arc? Although thousands of mRNAs are known to be transported into neuronal dendrites, very little is known about how and why different mRNAs respond differently to the same stimuli and why different mRNAs are regulated either on the level of their transcriptional or translational activity or in their positioning in

the neuron. Live imaging and tracking of single diverse mRNAs will demonstrate unique and convergent transport mechanisms of mRNAs and perhaps expose regulatory mechanisms of transport that conform to the unique function of the mRNA being observed.

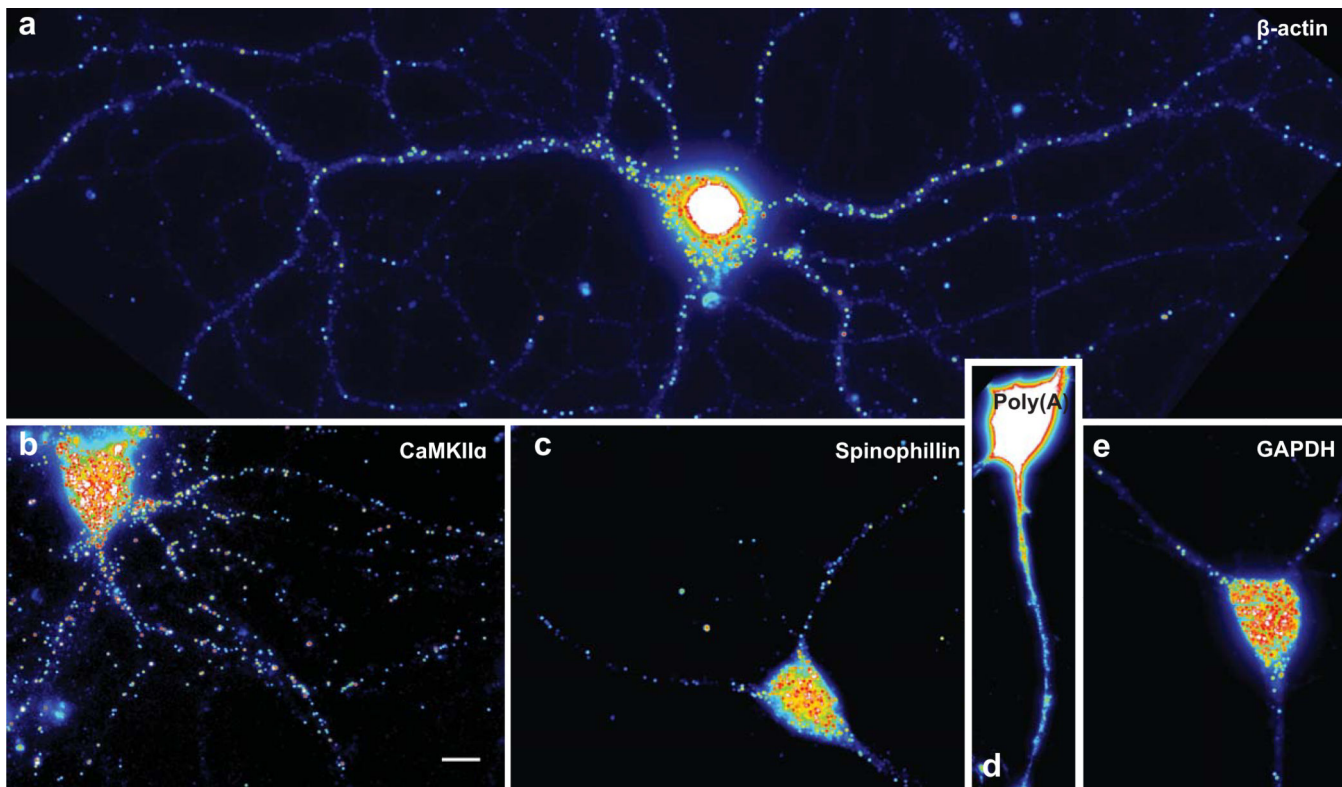


Figure 1. mRNA distribution in neurons varies widely

a. Single molecule FISH of endogenous β -actin mRNA in cultured primary hippocampal neurons demonstrates that the mRNA localizes along neurites. **b.** SmFISH of CaMKII α mRNA in cultured neurons. **c.** SmFISH of Spinophilin mRNA localized in neurites. **d.** FISH using dT sequences complementary to Poly(A) demonstrate the total amount of mRNA in dendrites. **e.** Visualization of endogenous GAPDH mRNA with smFISH shows that the mRNA is largely restricted to the soma. The mRNA signal was dilated by 2 pixels to aid viewing. The heat map represents the relative intensity. Bar, 10 μ m.

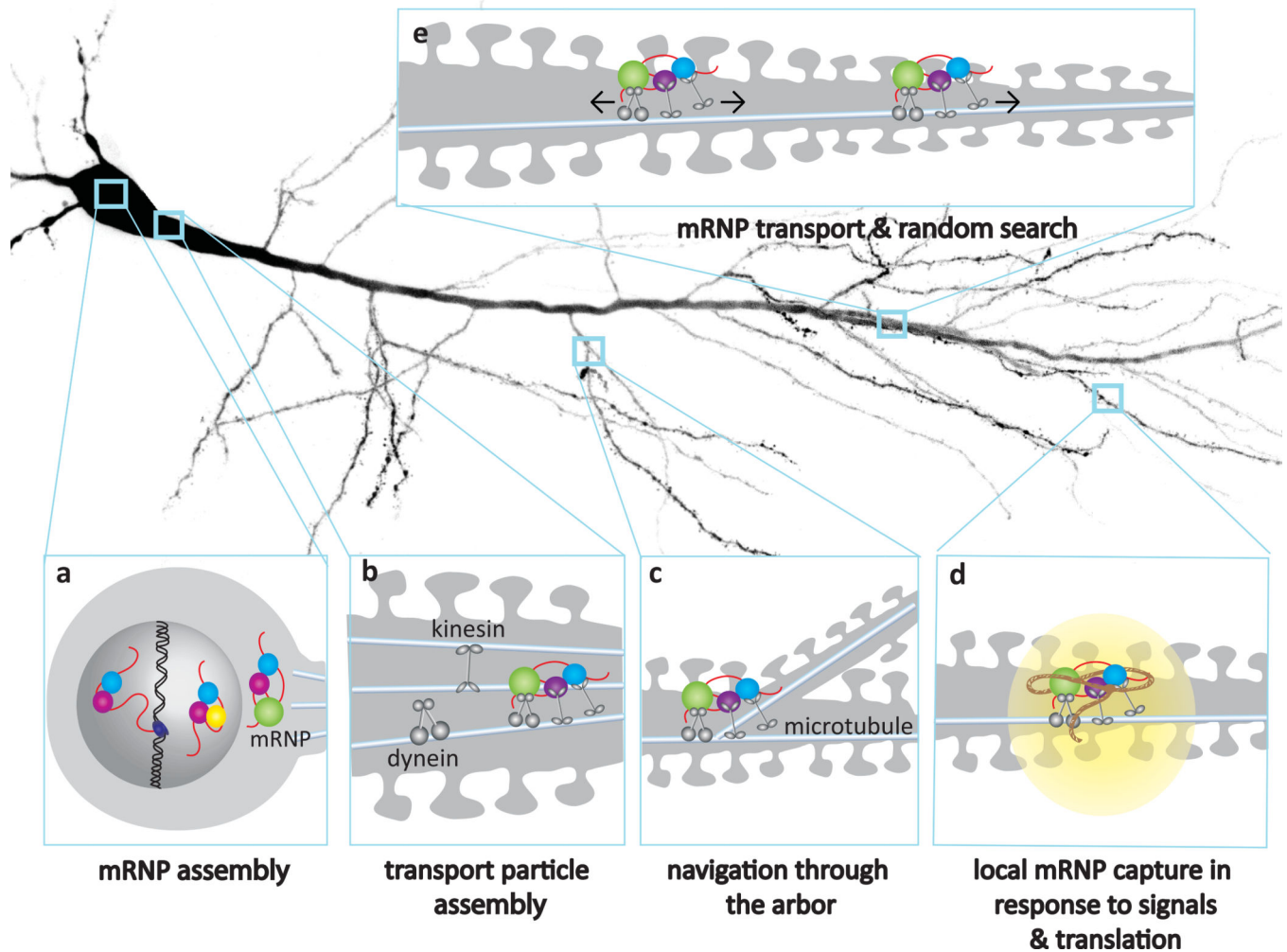


Figure 2. The life of a localized mRNA in a neuron

a. During, or shortly following synthesis, mRNAs are recognized and bound to RNA Binding Proteins (RBPs), which play a role in all aspects of the mRNAs travels and life. **b.** In neurons, a large proportion of mRNAs are actively transported. Although it remains unclear where precisely the mRNA binds motors, it is possible that assembly of the transport machinery occurs at or near the soma since many mRNAs can be seen actively transported in regions near the soma. **c.** Navigation of mRNAs through the arbor is a poorly understood process. Whether transport is random or biased towards specific branches or regions is unknown. Nevertheless, mRNA transport must account for the highly branched morphology of a neuron. **d.** mRNAs can be captured or stalled at specific regions in response to local activity. This may allow for the specific targeting of proteins to particular regions or synapses through local translation. **e.** mRNA imaging highlights the oscillatory behavior of actively transported mRNAs, reminiscent of random searching. This could possibly be due to multiple opposing motors associated with the mRNP, bipolar orientation of microtubules or alternating binding between microtubules and actin filaments. Future work will reveal more on how this behavior is regulated and how random it is.