Review

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Single-molecule insights into mRNA dynamics in neurons

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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 a neuron and how its activity is regulated at the single-molecule level.

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 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, which RNAs are present and under which conditions, and how translation is regulated (reviewed in [2,11]). As mRNA labeling methods and optical sensitivity have improved, so has our understanding of these questions. Techniques to visualize single mRNAs in fixed and live cells deliver single molecule intracellular resolution of endogenous 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 detection of a specific signal is undefined. In live neurons, single-molecule approaches have expanded our ability to explore the precise mechanisms 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 mRNAs in neurons has resulted in novel and at times even reformed insights into how mRNAs are localized in the brain [15–17]. For simplicity, technologies that 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 RNAs with critical local roles. The hierarchal 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 regions. Early demonstration of *in situ* hybridization in hippocampal slices and cultured neurons distinguished mRNAs that were present in dendrites from those that 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 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

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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 using nanostring technology revealed that relative mRNA abundances varied in excess of 1000-fold [25]. The sensitivity of the assay demonstrated that many species of mRNA 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 coexpression of distinct mRNA species in a single neuron *in vivo* [28]. The presence of such a diverse population of mRNAs 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 transport 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 messenger ribonucleoprotein (mRNP) 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 contributions of targeting elements or RBPs to localization. For instance, quantification of the localization of reporter mRNAs with alternative Spinophillin dendritic targeting elements (DTEs) 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 proxies to reveal the 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 bidirectional transport in neurites punctuated by pauses in motion. Loss or mutation of RBPs, combined with live tracking, has also been informative regarding the contribution of individual RBPs to active transport. Amyotrophic lateral sclerosis (ALS)-associated mutations in TDP-43 altered the transport

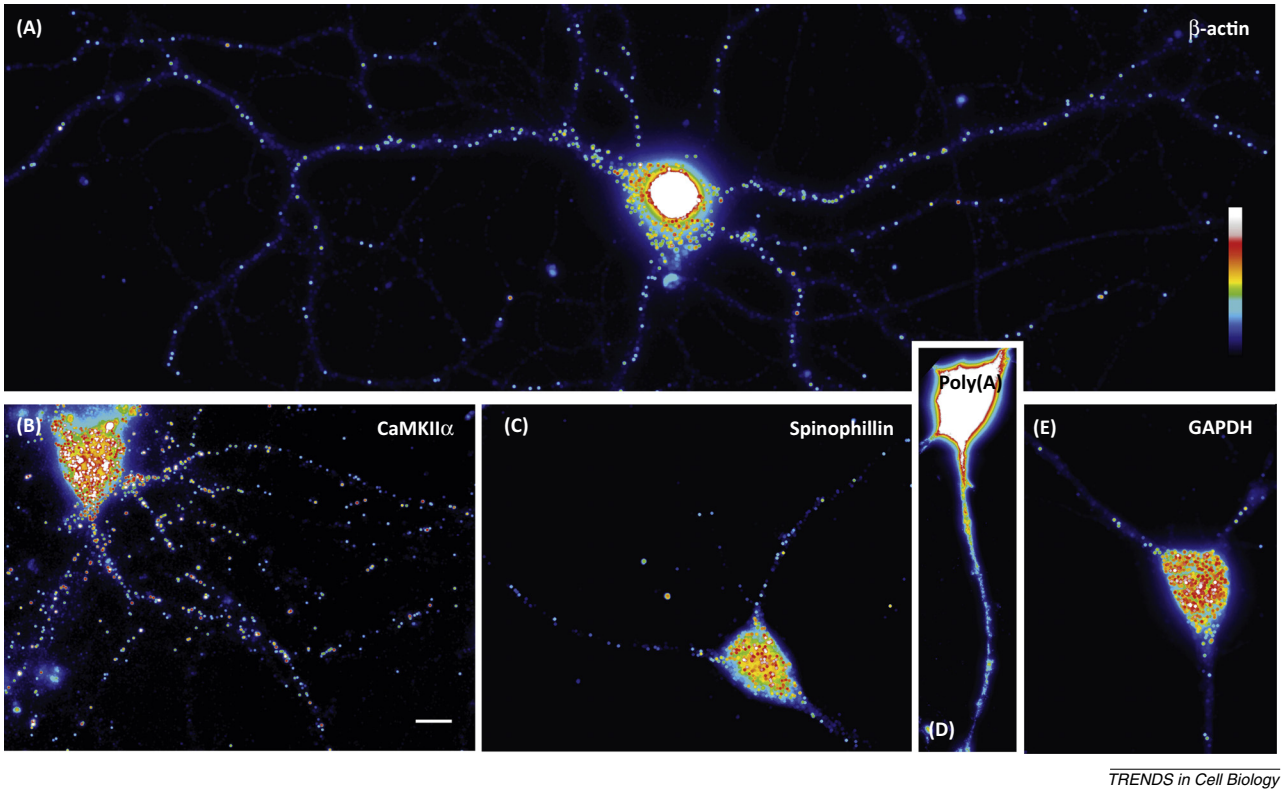

Figure 1. mRNA distribution in neurons varies widely. **(A)** Single-molecule fluorescence *in situ* hybridization (smFISH) of endogenous β-actin mRNA in cultured primary hippocampal neurons demonstrates that the mRNA localizes along neurites. **(B)** smFISH of CaMKIIα mRNA in cultured neurons. **(C)** smFISH of Spinophillin mRNA localized in neurites. **(D)** FISH using dT sequences complementary to poly(A) demonstrates the total amount of polyadenylated mRNA in dendrites. **(E)** Visualization of endogenous GAPDH mRNA with smFISH demonstrates that the mRNA is largely restricted to the soma. In all images, the mRNA signal was dilated by two pixels to aid viewing. The heat map represents the relative fluorescence intensity. Bar, 10 μm. All images presented at the same scale.