Dendritic RNA Transport: Dynamic Spatio-Temporal Control of Neuronal Gene Expression

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Introduction

Dendrites are master integrators of information flow in the brain and the sites of postsynaptic structural modifications that regulate the plasticity of synapse strength in response to neurotransmission. Recent data suggest that dendrites are also an important site for the dynamic regulation of localized gene expression in the nervous system, with subsets of synapses possessing the ability to independently alter synapse strength through the local synthesis of proteins. An important component to this regulation is the active transport of a select group of messenger RNAs (mRNAs) from the cell body into dendrites and targeting of these mRNAs to specific translation sites. Since several long-lasting forms of plasticity require protein synthesis, synaptic stimulation appears to be an important mechanism for the regulation of mRNA trafficking within dendrites.

Discovery of Dendritic mRNA Transport

Early speculations about the nature of synaptic connections led to the idea that cytoskeletal proteins played an important structural role in neuronal function, and the identification of translational machinery in dendrites but not axons by ultrastructural methods and actin protein localized to dendritic spines spawned an era of investigation into the nature of localized gene expression that is still ongoing. As it was found that microtubule-associated protein 2 (MAP2) was expressed selectively in the dendritic compartment, markers for neuronal polarity became the subject of great curiosity. Subsequent work tracked total mRNA movements in cultured neurons by pulse labeling with radioactive nucleotides to visualize time-dependent migration of mRNA into dendrites and showed the asymmetric localization of cytoskeletal mRNAs to dendrites, such as MAP2. More recently, eloquent molecular approaches have demonstrated the presence of numerous diverse mRNAs by directly aspirating cytoplasm from transected dendrites and identification by polymerase chain reaction. These studies served as the foundation for the idea that many regulatory and cytoskeletal mRNAs may be selectively transported into neuronal dendrites on microtubules and subsequently activated for translation at synapses in response to neuronal stimulation, an idea that currently has enormous support and has led to breakthroughs in visualizing specific transcript motility in living cells.

Structure and Composition of Dendritic Transport mRNPs

mRNAs are transported into dendrites in the form of large, electron-dense RNA- and protein-containing granules. Since these transport granules are distinct from stress granules, they will be referred to as transport messenger ribonucleoproteins (mRNPs). Neuronal cytoplasmic transport mRNPs appear quite heterogeneous in size and have been estimated by ultrastructural methods to be between 200 and 600 μm in diameter and greater in mass than polyribosome complexes (>500 S). They are formed initially when nucleocytoplasmic shuttling RNA-binding proteins (RBPs) associate with nascent transcripts in the nucleus to prevent premature ribosomal loading and translation and to aid in nuclear export of mRNAs. While the composition of proteins in transport mRNPs may change on exit to the cytoplasm, exchanging factors specific to export, for example, several RBPs appear to remain associated with transcripts until they are delivered to distal dendritic sites for translation. Numerous biochemical and proteomic experiments suggests that these cytoplasmic neuronal transport mRNPs are not homogeneous in composition, containing many different RBPs, such as Staufen, zipcode-binding protein 1 (ZBP1), heteronuclear RNP-A2 (hnRNP-A2), Pur-alpha and the fragile X mental retardation protein (FMRP), as well as many mRNA species, such as calcium/calmodulin-dependent protein kinase II (CaMKII)-alpha, activity-regulated cytoskeletal-associated (Arc), beta-actin, and the noncoding BC1. Included in the hundreds of diverse mRNAs that have been isolated from dendrites biochemically are several subunits of glutamate receptor subtypes and translational machinery components, such as ribosome subunits and elongation factor 1a. In addition, transport mRNPs contain multiple cytoskeletal motors such as conventional kinesin (Kif5) and dynein, which contribute to plus end- and minus end-directed transport along microtubules within dendrites. While the protein content may be diverse, the RBPs in transport mRNPs all appear to be important for repression of translation of mRNAs until reaching their distal site, where they are derepressed by regulatory signals. Both biochemical work and ultrastructural work have shown that they contain many densely packed ribosomes but are not translationally

competent as they lack proteins essential for initiation (e.g., eIF4). It is interesting to note that these mRNPs are dramatically remodeled on neuronal depolarization, presumably releasing ribosomes and mRNAs into local active translational complexes. The composition of transport mRNPs suggests that they represent motile pretranslation units that undergo dynamic remodeling in response to signals that induce their dendritic transport and subsequent translation.

Sequence and Structural Characteristics of Localization Signals in Neuronal Transport mRNPs

A unifying theme in the analysis of many localized mRNAs is the requirement for a *cis*-acting sequence for targeting the transcript to specific sites, or 'zipcodes.' These zipcodes are mostly localized to the 3′ untranslated regions (UTRs) of transcripts, where they are bound by *trans*-acting RBPs involved in the localization process (see Table 1). The zipcodes may contain several units that function in a multistep pathway to mRNA localization. One example is the localization of myelin basic protein (MBP) mRNA to the myelin-rich oligodendrocyte compartment. This is a two-step process involving an 11 nt element (the RNA transport signal, RTS) sufficient to direct a reporter mRNA into processes and a distinct 342 nt element (the RNA localization region) required for MBP mRNA targeting to or retention at the myelin-specific subcompartment in oligodendrocytes. The 11 nt RTS has been shown to mediate granule formation and dendritic trafficking with hnRNP-A2 in primary hippocampal cultures as well; however, a requirement for this 11 nt sequence in the transport of the native MBP mRNA has not been established. It is interesting to note that a conserved version of the RTS has also been found in several other dendritic mRNAs, such as MAP2, CaMKII-alpha, Arc, and γ-aminobutyric acid-A-receptor subunit and therefore may play an important role in mRNA targeting using similar machinery in neurons. However, the fact that a 640 nt element of MAP2 mRNA 3′UTR was found to be necessary for dendritic targeting suggests that there may be additional required elements present in the 3′UTR that control localization or stability. Another example of a bipartite element is the beta-actin mRNA, which contains both a 54 nt and a 43 nt zipcode, required for efficient localization to neuronal processes and growth cones. While some zipcodes in localized mRNAs such as beta-actin are necessary for localization of the native mRNAs, most zipcodes have been tested using reporter mRNAs, and therefore their requirement in the localization of native mRNAs is not known. Future work using genetic deletions or antisense technology can more directly address these shortcomings.

Some mRNAs, such as *bicoid* in *Drosophila*, may contain zipcode elements that are repeated several times in the 3′UTR to effect a more efficient localization as the deletion of one or more redundant elements is not sufficient to completely abolish targeting but rather diminishes the amount of mRNA localized over time. This is a feature shared by the beta-actin mRNA 3′UTR zipcode, which has a consensus sequence of ACACCC repeated twice within one zipcode element (the 54 nt chicken zipcode). Although some zipcodes may be conserved between mRNAs, there is no clear indication yet that the same proteins are involved in their localization. Rather diverse collections of RBPs have been found to associate with transcripts that have conserved zipcodes. This has inspired a thorough analysis of secondary structural features in zipcodes that may be more important than primary sequence conservation

Table 1 RNA-binding proteins and their corresponding *cis*-acting sequences in brain

RNA-binding protein	*mRNA*	*Sequence and location*	*Required for localization*[a]
ZBP1	beta-actin	ACACCC, within 54 nt zipcode in 3′UTR	+
hnRNPA2	MBP	GCCAAGGAGCC, 3′UTR	
	MAP2	GCCAAGGAGUC, coding	
CPEB	CaMKII-alpha	UUUUUUAUU X 2 (separated by 82 nt), 3′UTR	
HuD	tau	240 nt, 3′UTR	+
MARTA1/2	MAP2	640 nt, 3′UTR	
Unknown	CaMKII-alpha	3.0 kb, 3′UTR	+/−
		94 nt, 3′UTR	

[a]Plus sign (+) indicates *cis*-acting sequences shown to be required for full-length (either endogenous or exogenous) mRNA localization in dendrites either by deletion of element or using antisense to the endogenous element; no entry symbolizes that the *cis*-acting elements were tested only for localization of a heterologous reporter mRNA; +/− indicates conflicting reports for this mRNA (one study indicated that this 94 nt sequence can localize a heterologous reporter to dendrites (−), and another showed significantly diminished dendritic localization of this endogenous mRNA with a 3.0 kb deletion in the 3′UTR that left the 94 nt sequence intact (+)). CaMKII-alpha, calcium/calmodulin-dependent protein kinase II; CPEB, cytoplasmic polyadenylation element binding protein; hnRNPA2, heteronuclear ribonucleoprotein-A2; HuD, one of the embryonic lethal abnormal vision family of RNA-binding proteins; MAP2, microtubule-associated protein 2; MARTA, MAP2-RNA *trans*-acting protein; MBP, myelin basic protein; UTR, untranslated region; ZBP1, zipcode-binding protein 1.

since RNA can assume diverse three-dimensional structures and the 3′UTRs can possess structure without interfering with translation. Indeed many zipcodes appear to contain stem–loop structures where the essential sequences present binding sites for RBPs, as has been shown for ZBP1 binding to the 54 nt zipcode in beta-actin mRNA and for Staufen binding to the 3′UTR of *bicoid* mRNA. It is important to note that individual nucleotide changes that conserve the stem hydrogen bonding structure do not alter binding to the *trans*-acting RBPs or localization of the mRNAs whereas nucleotides that do disrupt the structure diminish binding and localization. A more thorough analysis of structural motifs common to localized mRNAs, may reveal a class of conserved structures present within mRNAs that share *trans*-acting RBP recognition sites. Indeed more-detailed structural information may explain how the dendritic targeting element (DTE) of MAP2 mRNA is recognized by the diverse RBPs Staufen, hnRNP-A2, MAP2-RNA *trans*-acting protein (MARTA)1, and MARTA2, or how MARTA1 recognizes both the DTE and the zipcode of beta-actin mRNA, which show little primary sequence homology.

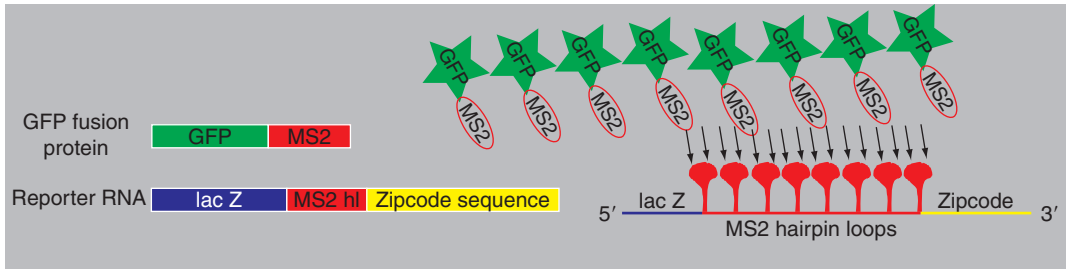
Trans-Acting Factors Involved in Dendritic mRNA Transport

The persistent association of RBPs with mRNAs from birth at the transcription site until death after mRNA translation and degradation suggests that RBPs are critical to the spatiotemporal regulation of mRNA expression. At least 300 RBPs are expressed in vertebrate brain during early developmental stages, defined by the presence of conserved RNA-binding motifs, such as the K-homology domain of hnRNP-K family of RBPs. Other common RNA-binding motifs include the RGG-box, the RNA-recognition motif, and the double-stranded RNA-binding domain. These RBPs usually have nuclear shuttling domains (nuclear export and import) to facilitate entry and exit from the nucleus, where they first associate with transcripts in their journey to distal sites for regulated translation. An important theme is that the mRNA must be translationally repressed by these RBPs to facilitate temporally and spatially localized gene expression and that this repression must be linked to the process of mRNA transport. Therefore RBPs may function to repress translation and initiate assembly of the transport-competent higher-ordered mRNP granules before being recognized by the transport machinery. This idea is supported by the observation that exogenous MBP mRNA injected into oligodendrocytes induces the formation of larger RNP particles before their transport into processes. Several RBPs involved in the regulation of mRNA transport and translation in neurons, such as cytoplasmic polyadenylation element binding protein 1 (CPEB1), ZBP1, and Staufen, have been implicated in this dual functional role already.

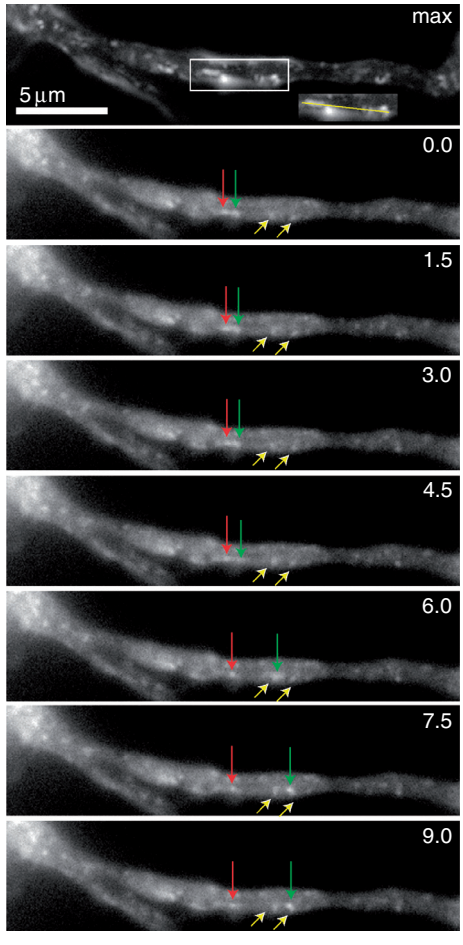
While a causal role for RBPs in the transport and translational regulation of dendritic mRNAs appears concrete, there is surprisingly little direct evidence for an essential role of these proteins in RNA localization (see Table 1), most likely because many of these proteins may play an essential role in early development and therefore knockout animals may not be viable. However, a viable knockout mouse has been generated for CPEB1, and neurons from these mutant mice have shown reduced dendritic localization of a reporter mRNA containing cytoplasmic polyadenylation elements (CPEs) within the 3′UTR. The absence of data demonstrating a reduction in the dendritic localization of endogenous mRNAs containing CPEs suggests that several RBPs, such as the other recently added members to the CPEB family of RBPs, may play a role in targeting different mRNAs containing CPEs to dendrites along with CPEB1. Alternatively, distinct RBPs may solely regulate specific stimulus-induced pathways for mRNA localization, as has been shown for the neurotrophin-induced localization of beta-actin mRNA to neurites and growth cones. This localization requires the beta-actin mRNA zipcode recognition by ZBP1 because uncoupling of the mRNA from ZBP1 using antisense oligonucleotides that are complementary to the zipcode has caused a reduced localization of beta-actin mRNA by this stimulation paradigm. Other proposed functions for RBPs in dendritic transport include a role for connection of the mRNA to molecular motors, such as that proposed for CPEB. The trafficking of MBP mRNA by hnRNP-A2 in oligodendrocytes and CaMKII-alpha mRNA in hippocampal neurons was reduced by antisense oligonucleotide knockdown of conventional kinesin (Kif5). Recent proteomic approaches have identified numerous RBPs in complex with this form of kinesin from brain extracts, including proteins already implicated in RNA localization: Staufen, Pur-alpha, and hnRNP-U. However, the molecular evidence for a connection to kinesin is still lacking, and further work is needed to understand the mechanism behind this connection, as well as connections to the minus end-directed microtubule motor dynein that contributes to the oscillatory and retrograde trajectories of transport mRNPs.

Dynamic Regulation of Transport mRNP Motility

Early studies in the transport of mRNAs to dendrites showed that on synaptic stimulation, immediate early genes such as Arc are turned on, and the mRNAs are rapidly transported into dendrites. This transport



a



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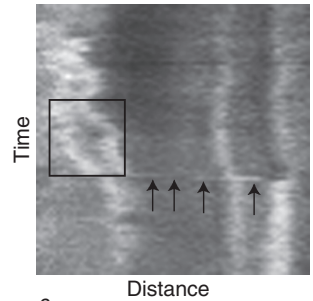

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Figure 1 Analysis of rapid dendritic transport of calcium/calmodulin-dependent protein kinase II (CaMKII)-alpha messenger RNA (mRNA) in living hippocampal neurons. Cultured mouse hippocampal neurons (10 days *in vitro*) were transfected with the indicated constructs and imaged 18 h later using epi-fluorescence microscopy with filter sets for green fluorescent protein (GFP; a 100×, 1.35 numerical aperture lens was used here) and a closed-chamber heated coverslip apparatus (Bioptechs, USA). (a) Schematic of constructs used to visualize CaMKII-alpha mRNA in living cells. One construct is a fusion of GFP to the viral protein MS2 (GFP fusion protein) and contains a nuclear localization sequence. The second construct is the reporter mRNA, which consists of a fusion of the lac Z coding region (lac Z) to 8 repeats of the MS2-binding hairpin loops (MS2 hl) and the zipcode sequence, which here is the 3′UTR of murine CaMKII-alpha (both the MS2 loops and the zipcode are in the 3′UTR of this construct). Since the MS2 hairpin loops each contain two binding sites for MS2 protein, each mRNA can theoretically be linked to 16 GFP molecules. (b) Analysis of CaMKII-alpha reporter mRNA movements in neurons using Image J. A time-lapse movie stack was converted to a maximum projection image (max), which displays the maximum pixel for each frame with stationary particles as dots and moving particles as lines, highlighting their trajectories over the sequence. A dendritic region of interest (ROI) was highlighted (white box) for further analysis (see **Figure 1(c)**, kymograph), and the line used to analyze a particle trajectory was drawn over that ROI (yellow; see inset). Still frame images of the highlighted particle movements are shown in sequence with the corresponding time stamps (every 1.5 s; upper right corners). An mRNA particle emerges from a stationary particle (red arrow) and traverses another stationary particle (green arrow), which then moves rapidly in the anterograde (right; cell body is at left) direction together with the previously moving particle. The particles move in parallel toward two stationary particles positioned anterograde in the dendrite (yellow arrows), then traverse the first stationary particle (left yellow arrow) and come to a stop at the second, more distal particle (right arrow), where they remain throughout the duration of the imaging series. (c) A kymograph of the rapid CaMKII-alpha

requires *N*-methyl-D-aspartate (NMDA) stimulation but not protein synthesis, and activation of dendritic domains in the medial dentate gyrus by specific stimulation of the medial entorhinal cortex caused a selective increase in Arc mRNA to those sites, implying input-specific localized synaptic activation and subsequent delivery of mRNA to those synapses. This increase in trafficking to dendrites is thought to require the cytoskeleton and interaction of the transport mRNPs with anterograde molecular motors. How the mRNAs recognize the sites at which they are anchored remains unknown, although there is work from other systems such as *Drosophila* that shows a role for microtubule-based motors in anchoring mRNAs. However, given the abundant actin-rich regions surrounding the synapse, one may speculate that myosin motors may also be involved in the anchoring process. In fact, recent data supports a role for MyoVa in RBP movement into dendritic spines.

Neuronal stimulation can cause the localization of mRNAs for neurotrophic factors themselves, such as brain-derived neurotrophic factor (BDNF) and its receptor kinase mediator trkB in cultured neuronal dendrites. In addition, stimulation can enhance the extent of localization, with BDNF and trkB mRNAs enriched only in the proximal dendrites under basal conditions, and upon depolarization, a significant fraction of mRNAs move into the distal dendrite. NMDA and neurotrophin stimulation results in an increase of ZBP1 and beta-actin mRNA localization to dendrites of cultured neurons. With an emerging technology of *in vivo* labeling of mRNAs using green fluorescent protein (GFP)-tagged MS2 reporter construct fusions (see **Figure 1**), rapid analysis of specific transport mRNP dynamics in living neurons became possible. Particles moved with both oscillatory and persistent trajectories, both in the anterograde and retrograde directions, and neuronal activity modified these dynamics. Neuron-wide depolarization (using KCl) induced the net anterograde transport of CaMKII-alpha mRNA in dendrites and caused a repositioning of the population of mRNAs with respect to synapses for those mRNAs already localized to dendrites. In addition, high-frequency stimulation leads to the rapid increase in CaMKII-alpha mRNA in synapse fractions isolated from dentate gyrus, a process independent of NMDA activation. The results suggest that a fine-tuning mechanism may exist to regulate the local population of mRNAs in shafts and spines, modifying the content of proteins present within those synapses on stimulation in order to effect long-term changes in synapse plasticity. Consistent with this idea, the localization of RBPs involved in plasticity, such as FMRP, is altered in dendrites on stimulation. Both the protein and the mRNA encoding FMRP are trafficked into dendrites of mature hippocampal cultures in response to stimulation of group I metabotropic glutamate receptors (mGluRs). Similarly, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunit mRNAs are increased in their dendritic localization by mGluR, but stimulation of NMDA causes a selective decrease in the abundance of dendritic AMPA subunit mRNAs. This suggests that activity can bidirectionally control the relative abundance of excitatory ionotropic receptor mRNAs within the vicinity of synapses, possibly contributing to changes in synaptic efficacy during developmental plasticity.

Function of Dendritic mRNAs in Synapse Plasticity and Neuronal Polarity

The first evidence of a specific requirement for dendritic mRNA targeting in mediating synaptic plasticity came from studies on CaMKII-alpha mRNA. This mRNA harbors a zipcode within the 3′UTR that is sufficient for dendritic localization of reporter constructs bearing this zipcode. In a mouse that contained a deletion of most of the 3′UTR in the endogenous CaMKII-alpha allele, physiological experiments showed defects in long-term potentiation at synapses, and behavioral experiments showed alterations in several tests for spatial and contextual conditioning that are hallmarks for learning and memory. The neurons from these mice showed significantly diminished CaMKII-alpha protein levels at synapses, suggesting that targeting of the mRNA to dendrites is essential for local protein expression to support long-term plasticity. What remains unclear is at which stage during brain development the requirement for this local synthesis occurs. Also, other studies have indicated that the 5′-most 94 nt of the 3′UTR sequence that was left intact in the above-mentioned deletion mutant was sufficient to mediate dendritic localization of reporter constructs into

mRNA particle trajectory outlined in (b) (max inset, yellow line), where distance moved (in pixels, *x*-axis) is plotted against time (seconds, 1.5 s interval per line). This analysis shows stationary particles as vertical lines and moving particles as nonvertical with velocities equal to the slopes of the lines. An example of the calculation of trajectory velocities is shown (black box) for two particles moving in parallel within the dendrite. These particles appear to merge and then move rapidly (black arrows) toward several other stationary particles, traversing the first stationary particle and then stopping at the second stationary particle. The increased intensity of the right-most vertical line (second stationary particle) after the moving particle stops there confirms this event.

dendrites, suggesting that reporter studies may give misleading results. However, it is interesting that associates with translational regulator CPEB, an RBP that associates with CaMKII-alpha mRNA, plays a role in the modification of synaptic plasticity and is itself phosphorylated by the CaMKII-alpha enzyme. This feedback loop regulation may be an important paradigm for mechanisms that underlie activity-induced changes in synapse strength.

Fragile X syndrome (FXS) is a neuronal disease characterized by mental retardation and spine dysgenesis due to loss of function of FMRP, a key RBP thought to regulate translation of mRNAs in dendrites. Physiological experiments suggest that one form of long-term depression, which depends on mGluR and rapid protein synthesis, is altered in mouse hippocampus bearing a null-mutation for FMRP. Therefore the control of dendritic mRNA processing (transport, translation, or stability) by FMRP may be important for this form of plasticity and may link changes in plasticity to dendritic morphological transformations that are fine-tuned during development. Indeed one of the mRNAs regulated by FMRP is MAP1b, an important cytoskeletal protein that may contribute to the phenotypic alteration of dendritic protrusions that are hallmarks of mental retardation in general and FXS specifically. Another RBP in brain, Staufen2, appears to be involved in regulation of dendritic mRNA transport and causes reduced dendritic spine numbers with concomitant increases in immature-like protrusions when its expression is reduced in neurons. It is interesting to note that one of the target mRNAs that is reduced in dendrites of Staufen2-depleted neurons is beta-actin. The delivery of beta-actin mRNA into dendrites is linked to the growth of dendritic filopodia and formation of filopodial synapses in response to BDNF, a process that requires ZBP1 and its interaction with the zipcode of beta-actin mRNA. These studies implicate a role for transport mRNPs and the delivery of cytoskeletal-encoding mRNAs to dendrites in the regulation of morphological plasticity during neuronal development.

A role for mRNA delivery in establishment of neuronal polarity is under investigation. Two major candidate mRNAs, MAP2 in dendrites and tau in axons, are found in these specific subcellular compartments exclusively, and their local translation there may be important for establishment and maintenance of neuronal polarity. These mRNAs are bound by distinct RBPs that are transported with these mRNAs in dendrites and axons: MAP2 by the MARTA1/2 proteins and tau by one member of the embryonic lethal abnormal vision family of RBPs, HuD. While the molecular mechanism of their differential transport is not well characterized, the details are beginning to emerge. MAP2 mRNA contains a 640 nt zipcode, the DTE mentioned earlier, which is sufficient to confer localization of a reporter mRNA to the dendritic compartment of neurons. An 11 nt sequence within the coding region of MAP2 mRNA has been suggested as a targeting element for localization of a GFP reporter mRNA to oligodendrocyte processes and hippocampal dendrites; however, a reporter mRNA containing the entire coding region of MAP2 is not able to localize efficiently in neurons, suggesting that this element may not be sufficient for dendritic targeting of endogenous transcripts and that inhibitory elements within other regions may serve to regulate transport.

Tau mRNA contains a 240 nt zipcode that is required for targeting to the axonal compartment of differentiated P19 cells that segregate dendritic and axonal markers similarly to primary neurons. This zipcode is also sufficient to redirect the dendritic MAP2 mRNA to axons when substituted for the MAP2 3′UTR. Conversely, the dendritic 640 nt zipcode located in the 3′UTR of MAP2 mRNA is sufficient to redirect tau mRNA to dendrites when substituted for the tau 3′UTR, suggesting that these zipcodes are primary determinants of localized gene expression in the establishment of neuronal polarity. Other factors that may contribute to the distinct localization of MAP2 and tau mRNAs include the association of these transport RNPs with two distinct plus end-directed microtubule motors, Kif5 and Kif3, respectively. Future work may reveal how the localized expression of these genes maintains segregation of downstream determinants of neuronal polarity.

Dendritic mRNP Transport and Neuronal Disease

Several diseases of the nervous system have been attributed to defects in the function of RBPs and in the processing of RNA. FMRP is an RBP highly expressed in brain that is silenced in FXS, the leading cause of inherited mental retardation in humans and a known cause of autism. FMRP binds to numerous mRNAs in brain to repress translation, and loss of FMRP results in elevated mRNA translation and excessive mGluR-dependent long-term depression of synaptic strength in the hippocampus and cerebellum of a mouse model of FXS. In addition, neurons lacking FMRP show an increase in the number and length of dendritic protrusions. Although several identified mRNA targets of FMRP are localized to dendrites and have important functions in neuronal homeostasis, the mechanisms that give rise to altered dendritic morphology and plasticity are not well understood. Similarly, translin is an RBP that is expressed in brain

and regulates mRNA transport and translation in dendrites. Mice lacking translin show altered expression of several mRNAs in brain and have impaired learning and memory function in behavioral tests. One target mRNA of translin is CaMKII-alpha, and since its dendritic transport has been shown to be critical for normal developmental plasticity, this aspect may be altered in neurons lacking translin.

Another RBP is the spinal motor neuron (SMN) protein that is not expressed in the progressive neurodegenerative disease spinal muscular atrophy. SMN functions in the assembly of small nuclear RNP complexes that participate in RNA splicing, and recent work suggests that SMN may play a role in the localization of mRNP complexes to neuronal processes as well. Nova proteins also function in the splicing of neuronal mRNAs, and the autoimmune disorder paraneoplastic opsoclonus myoclonus ataxia (POMA) results in the antagonism of Nova RBP function. One important target of Nova is the inhibitory glycine receptor, and the misregulated splicing of this mRNA may contribute to the excessive motor function in POMA patients. It is interesting to note that Nova-null mice are deficient in a novel form of synaptic plasticity that may result from defective splicing, long-term potentiation of the slow inhibitory postsynaptic current. The localization of Nova target mRNAs in neurons lacking this RBP remains to be determined. Together these alterations in mRNA processing and expression underlie the important function of localized gene expression in the nervous system for the maintenance of synaptic plasticity and normal signal transmission.

Analysis of mRNA Dynamics in Living Neuronal Dendrites

Recent advances in molecular techniques that aid in the visualization of mRNA movements in living neurons have led to the ability to tag mRNAs with fluorescent fusion proteins such as GFP. By insertion of a viral hairpin repeat sequence in the 3′UTR of a given mRNA, one can co-express a GFP fusion containing a small protein, MS2, that binds to these hairpin repeats with nanomolar affinity. With this system, one can follow the movements of a CaMKII-alpha reporter mRNA containing the complete 3.2 kb 3′UTR shown to be sufficient for dendritic targeting in neurons, when fused to eight MS2 hairpin loops. The same system has been used to analyze the transport of CaMKII-alpha reporter mRNAs in living hippocampal neurons with improved camera sensitivity, enabling faster sampling rates (Figure 1).

The analysis of one movie shows several transport mRNP particles (herein termed ‘particles’) moving rapidly in the anterograde and retrograde directions, as well as stationary and oscillatory particles, with an obvious heterogeneity in the intensity of both moving particles and stationary ones, suggesting that the number of mRNAs in these particles varies over a considerable range. To analyze the velocities and trajectories of these particles, the program ImageJ, which is a software that runs on most computer platforms and is available at no cost from the National Institutes of Health, may be employed. Using this program, one can readily identify moving particles by first generating a maximum projection of a time-lapse stack of images (Figure 1(b)). This analysis shows stationary particles as dots and motile particles as lines. The trace of one trajectory with reslicing generates a kymograph that displays time (sequential frames in the stack) on the *y*-axis and distance (pixels) on the *x*-axis (Figure 1(c)).

One can notice from the kymograph that the trace line reveals two particles moving in parallel (within the black box) at the same velocity that join together with a stationary particle to form a larger particle (Figure 1(b), red arrow). Then one of these particles rapidly moves out of the stationary particle, almost unnoticeable on the still frame analysis panel (Figure 1(b), green arrow) but very distinct on the kymograph, where it shows a long, almost horizontal line (Figure 1(c), black arrows) that traverses another stationary particle (Figure 1(b), left yellow arrow) and comes to a stop at a second stationary particle (Figure 1(b), right yellow arrow). Overall, this analysis showed that persistent particles containing the CaMKII-alpha reporter mRNA moved at $0.8\,\mu m\,s^{-1}$ on average with a maximal rate of $2.1\,\mu m\,s^{-1}$. In comparison with previous work, this faster sampling rate reveals that transport mRNPs move at rates at least tenfold faster (previous average velocities were estimated at $0.05–0.10\,\mu m\,s^{-1}$ and maximal velocities at $0.1–0.2\,\mu m\,s^{-1}$). It also reveals that many trajectories involve pauses or oscillatory movements that contribute to lower average velocities when sampling is done at lower frequencies. Future work may determine whether classes of transport mRNPs move at homogeneous rates and how neuronal activity may differentially modulate these velocities within dendrites.

See also: Adult Cortical Plasticity; Axonal mRNA Transport and Functions; Axonal and Dendritic Identity and Structure: Control of; Cytoskeleton in Plasticity; Dendrites: Localized Translation; Developmental Disability and Fragile X Syndrome: Clinical Overview; Fragile X Syndrome; Neuronal Plasticity after Cortical Damage; Plasticity and Activity-Dependent Regulation of Gene Expression; RNA Binding Protein Methods; Synaptic Plasticity: Neuronogenesis and Stem Cells in Normal Brain Aging.

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Relevant Website

http://rsb.info.nih.gov – ImageJ, Image Processing and Analysis in Java.