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Spatially restricting gene expression by local translation at synapses

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Abstract

mRNA localization and regulated translation provide a means of spatially restricting gene expression within each of the thousands of subcellular compartments made by a neuron, thereby vastly increasing the computational capacity of the brain. Recent studies reveal that local translation is regulated by stimuli that trigger neurite outgrowth/collapse, axon guidance, synapse formation, pruning, activity-dependent synaptic plasticity, and injury induced axonal regeneration. Impairments in the local regulation of translation result in aberrant signaling, physiology, and morphology of neurons, and are linked to neurological disorders. This review highlights current advances in understanding how mRNAs are translationally repressed during transport and how local translation is activated by stimuli. We address the function of local translation in the context of fragile X mental retardation.

Regulated translation at the synapse: function and molecular mechanisms

Stimulus-induced changes in gene expression are fundamental to the development and function of all organisms since they allow a shared genome to undergo specific patterns of expression to give rise to an extraordinary variety of distinct cell and tissue types. Within the nervous system, stimulus-induced changes in gene expression function to alter and refine circuit connectivity in a persistent manner. In this way, experience modifies our memories, behaviors, feelings and thoughts such that nature and nurture combine to determine who we are as individuals. Compared to non-neuronal cells, neurons, with their highly polarized morphologies, pose a distinct set of challenges, particularly in terms of the spatial regulation of gene expression. Neurons elaborate axonal and dendritic processes whose lengths often exceed the diameter of the cell soma by many orders of magnitude, and mature neurons can

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form up to 10,000 synaptic contacts. Stimulus-induced changes in structure and function can occur at each of these synapses, often as the result of regulated translation of localized mRNAs.

Early studies showing that ribosomes are predominantly restricted to the cell soma gave rise to a long-standing belief that all proteins were synthesized in the cell body, and then transported to distal sites. While the initial detection of ribosomes in dendrites [1] was met with some skepticism, it is now widely accepted that *local* protein synthesis occurs in neurons. Systematic, unbiased approaches have led to the identification of hundreds of localized mRNAs [2–5]. In invertebrate neurons [3,6], local translation of neuritically localized mRNAs underlies synapse formation [7], learning-related synaptic plasticity [8,9], and injury-induced changes in excitability [10]. In the developing vertebrate nervous system, mRNAs are detected in axonal growth cones, where they are translated during axon guidance and synaptogenesis [11–14] (see however Roche et al [15]). In mature vertebrate neurons, most studies have focused on dendritically localized transcripts whose translation is regulated by stimuli that produce changes in synaptic efficacy [16–18], although translation in axons of mature vertebrate neurons is known to be required for neural regeneration following axonal injury [19,20].

mRNA transport into dendrites has been shown to be mediated by *cis*-acting RNA elements typically contained within the 3'-untranslated region (UTR), which are recognized by *trans*-acting RNA binding proteins (see Box 1). These messenger ribonucleoproteins (mRNPs) combine with additional RNAs and proteins to form an RNA granule (see Box 2) that is actively transported by motor proteins along the cytoskeleton to its final destination [21,22]. Ongoing efforts in the field are aimed at understanding 1) how is the translation of mRNAs repressed during transport?; 2) how do activating stimuli regulate translation of specific transcripts once they have reached their destination?; and 3) how is local translation altered in diseased neurons, and what does this reveal about the function of local translation in the brain? In this review, we highlight recently gained insights into each of these questions, focusing on mRNA localization and regulated translation in mature CNS neurons (figure 1).

How are mRNAs silenced during transport?

To spatially restrict gene expression, mRNAs must be translationally repressed until they reach their final destination. Translational repression is usually achieved by binding of trans-acting factors that prevent the mRNA from being translated. Here we consider several of best characterized such factors, eukaryotic Initiation Factor 4E (eIF4E) binding proteins, Fragile X Mental Retardation Protein (FMRP), Cytoplasmic Polyadenylation Element-Binding Protein (CPEB), Pumilio and microRNAs. Although not yet demonstrated in neurons, RNA oligomerization and formation of large silent mRNPs can also efficiently suppress translation (see below).

Repression of cap-dependent translation initiation by eIF4E binding proteins

Eukaryotic translation, the process by which mRNA is translated into proteins, consists of three main steps: initiation, elongation and termination, and in the processes of neurons, is typically cap-dependent. Cap-dependent translation refers to the form of translation in which proteins known as eukaryotic Initiation Factors (eIFs) interact with the "cap" or 7-methyl-GTP structure located at the 5' end of the mRNA [23]. The initiation complex eIF4F is composed of eIF4E, the protein that directly binds the cap; eIF4G, which binds to Poly(A)-Binding Protein (PABP), a protein that bridges the 5' and 3' ends of the mRNA, and thereby promotes mRNA circularization, and eIF4A, an RNA helicase that unwinds secondary structures in the 5'UTR to allow scanning to the translation initiation site. The 40S ribosomal subunit is thought to be recruited to the mRNA by eIF3, which binds to eIF4G.

As might be expected for a complex biochemical process, the initiation phase of translation is a common target for regulation. Regulation of protein synthesis can occur by posttranslational modification of translation factors, which would modulate translation of an array of mRNAs, and/or binding of RNA regulatory proteins to the 5' or 3' UTRs of specific mRNAs. One of the best-characterized mechanisms involving posttranslational modification is that of mTOR-dependent phosphorylation of 4E-BP, an eIF4E binding protein. Unphosphorylated 4E-BP competes with binding of eIF4G to eIF4E, thereby blocking assembly of the eIF4F initiation complex and initiation of cap-dependent translation. In addition, some translational regulatory proteins such as Maskin, neuroguidin and CYFIP1, also inhibit cap-dependent translation through regulating binding of eIF4G to 4E. These 4E-BP-like proteins bind specific transcripts directly or indirectly *via* adaptor proteins, and in this way repress translation in a transcript-specific manner. mTOR-dependent phosphorylation of 4E-BP releases eIF4E, enabling it to assemble with eIF4G to form the initiation complex eIF4F.

In neurons, FMRP recruits the 4E-BP cytoplasmic FMR-interacting protein-1 (CYFIP1) to target mRNAs, repressing their translation [24]. The cytoplasmic polyadenylation element-binding protein (CPEB) associates with the 4E-BP neuroguidin and Maskin, and represses translation of a large set of CPE-containing mRNAs [25,26]. Recent work has indicated that a dendritically localized non-coding RNA, brain cytoplasmic (BC1) RNA, may regulate the interaction between CYFIP1 and FMRP [27] (but also see Iacoangeli et al [28]). Of note, BC1 also binds eIF4A, blocking its helicase activity [29].

Additional mechanisms of translational repression target later steps in translational initiation

Members of the Pumilio family of translational repressors are evolutionarily conserved RNA-binding proteins that regulate translation of specific mRNAs by binding their 3'-UTRs and promoting deadenylation [30]. In neurons, Pumilio regulates the morphology of presynaptic terminals at *Drosophila* neuromuscular junctions by repressing translation of eIF4E in postsynaptic structures [31], and is implicated in learning and memory, regulating the translation of synaptically localized mRNAs [32]. Pumilio2 has been detected as a component of RNA granules in dendrites of hippocampal neurons [33]. By analogy to yeast, these findings suggest that Pumilio family members may repress translation of localized mRNAs in neurons by blocking formation of the 80S ribosome.

Additionally, although not yet demonstrated in neurons, RNA oligomerization and formation of large silent mRNPs may also efficiently suppress translation of the mRNA encoding oskar, a protein required for the development of the posterior pole plasm in the *Drosophila* oocyte, perhaps by preventing accessibility of the translational machinery to the mRNA [34]. Of note, many components of the granule or RNP that contains oskar mRNA have been identified and seem to localize to neuronal processes, including polypyrimidine tract binding protein (PTB), barentsz and staufen [22,35].

microRNAs and transcript-specific translational repression

MicroRNAs (miRNAs) are non-coding, ~21 nt long RNAs whose mode of action involves complementary base pairing to sequences in a target mRNA, leading most commonly to translational repression of the target. Complementary binding can be partial and usually only involves the 6–8 nt long "seed site" in the miRNA. As such, each miRNA can repress the translation of a relatively large number of specific transcripts. Over half of all identified mouse miRNAs are expressed in the brain [36], and some of these are also present in dendrites and synapses [37,38]. Moreover, transcripts encoding synaptic proteins, including FMRP and PSD95, comprise the largest group of predicted targets of human miRNAs [39].

Together, these findings support the concept that miRNAs could regulate the translation of localized mRNAs in neurons [40]. Consistent with this hypothesis, Schratt and colleagues have shown that miR-134 colocalizes with and regulates translation of the mRNA encoding LIM kinase (LIMK), an actin-binding kinase that phosphorylates members of the ADF/cofilin family of actin binding and filament severing proteins, whose expression is implicated in spine morphogenesis [41]. In a more recent study, miR138 was also found to localize to dendrites, where it represses translation of the depalmitoylation enzyme APT1F and negatively regulates the size of dendritic spines [42]. Smalheiser and Lugli detected pre-miRNAs, as well as components of the miRNA processing machinery, in synaptoneurosomes [43]. In this study, the authors propose a model in which pre-miRNAs undergo activity-dependent processing into mature miRNAs at stimulated synapses to generate synapse-specific translational repression of transcripts.

How does local stimulation regulate the translation of localized mRNAs?

While translation of localized mRNAs is repressed during transport, it is likely that, upon arrival at their final destination, distinct stimuli and/or patterns of neuronal activity triggers the translation of specific subsets of localized mRNAs. How are local extracellular cues and stimuli transduced into local translational activation? How do growth cones and synapses decode the specific signal and activity patterns? Stimuli that activate mRNA translation do so both by regulating general components of the protein synthetic machinery and by regulating mRNA-specific repressors.

mTOR and S6K pathways release repression of general translation initiation

Diverse synaptic signals regulate the translation of localized RNAs. Such localized stimuli include those that trigger synapse formation, pruning, neurite outgrowth/collapse, activity-dependent synaptic plasticity and injury-induced axonal regeneration. A critical regulator of activity-dependent protein synthesis in dendrites is metabotropic glutamate receptor (mGluRs). mGluRs are G protein-coupled receptors enriched at excitatory synapses throughout the brain where they act to regulate glutamatergic neurotransmission. Group I mGluRs (mGluR1/5) activate phospholipase C, leading to intracellular Ca^{2+} mobilization, and the extracellular signal-regulated kinase (ERK)–mitogen-activated protein kinase (MAPK) pathway through which they modulate synapse-to-nucleus communication. Signaling by mGluR1/5 is critical to synaptic circuitry formation during development and is implicated in forms of plasticity including long-term potentiation (LTP), LTD, associative learning and cocaine addiction [44]. mGluR1/5 elicit synapse-specific modifications in synaptic strength and spine morphology by stimulating rapid local translation of dendritic mRNAs including *Fmr1*, which encodes FMRP [45].

The PI3 kinase/Akt (protein kinase B)/mammalian Target of Rapamycin (mTOR)/p70S6K and Ras/MAPK/p90S6K signaling pathways play central roles in activity-dependent local translation in dendrites and are critical signaling pathways downstream of group I mGluRs (figure 2) [46]. Both pathways target translational initiation by regulating the phosphorylation status of eIF4E and 4E-BPs. ERK/MAPK-dependent phosphorylation of eIF4E increases the rate of general cap-dependent translation, while mTOR-dependent phosphorylation of 4E-BPs decreases their affinity for eIF4E, releasing eIF4E, thereby promoting translational initiation[47–50]. Both pathways also induce phosphorylation of the ribosomal protein S6, which promotes translation of mRNAs with 5’ terminal oligopyrimidine tracts (5’TOPs), for example transcripts encoding ribosomal proteins. Note, however, that studies of a knock-in mouse with all S6 phosphorylation sites mutated revealed that S6 phosphorylation is dispensable for 5’TOP-dependent translation [51].

In neurons, components of both the PI3K/Akt/mTOR and Ras/MAPK/p90S6K pathways are present at synapses where they are activated by stimuli that promote protein synthesis-dependent forms of synaptic plasticity (figure 3). In both cases, these signaling pathways are thought to promote synaptic plasticity *via* regulation of local protein synthesis. ERK and mTOR activation are required for late-phase LTP (L-LTP) [47,48,52–54] and mGluR-dependent long-term depression (mGluR-LTD) [47,48,52–54].

Elongation factor eEF2 and regulation of translation elongation

Activity can also regulate local protein synthesis by targeting translational elongation. Elongation factor eEF2, which catalyzes the translocation of ribosomes along the mRNA, serves as a biochemical sensor for local translation *via* bidirectional activity-dependent phosphorylation [55]. Action potential-mediated network activity maintains eEF2 in a relatively dephosphorylated (active) state, whereas spontaneous neurotransmitter release (miniature synaptic transmission) promotes its phosphorylation and thus tonically suppresses local translation [55]. Following chronic silencing of activity, eEF2-becomes dephosphorylated (active) and local protein synthesis is triggered, which is required to rapidly increase synaptic expression of GluR1 and maintain synaptic homeostasis in the circuit [56].

During mGluR-LTD, which requires rapid *de novo* protein synthesis, eEF2 is phosphorylated by eEF2K, a Ca^{2+}/calmodulin-dependent kinase that binds to mGluR. The group I mGluR agonist DHPG releases eEF2K to phosphorylate eEF2 and thereby inhibits elongation of translation. While this attenuates translation of most transcripts, the translation of other mRNAs is facilitated. One such mRNA encodes the Activity-regulated cytoskeletal protein Arc [57,58]. Arc is encoded by an immediate early gene whose mRNA is rapidly transported into dendrites [59]. The Arc protein is required for internalization of AMPA receptors during LTD [58]. Its translation is increased during DHPG-induced LTD in an eEF2-phosphorylation-dependent manner [57,58].

Stimulus-induced translational activation by release of transcript-specific repressors

Mechanisms of translational activation that impinge on general translation factors, such as those described above, regulate the translation of many transcripts. Stimulus-induced modifications of transcript-specific translational repressors in contrast can regulate translation of specific mRNAs.

Shiina and colleagues [60] identified the protein RNA Granule Protein 105 (RNG105) as a component of RNA granules that functions as a translational repressor that is released from RNA granules following BDNF stimulation, concomitant with an increase in translation of localized mRNAs such as CamKIIα. As another example, β-actin mRNA translation in axonal growth cones is repressed by a protein that binds to the “zipcode” element in its 3’UTR, zipcode binding protein 1 (ZBP1) [61]. In growth cones, ZBP1 is phosphorylated by Src kinase, which releases the protein from the mRNA, and activates β-actin translation [61].

In the case of CPEB, synaptic stimulation switches its function from a translational inhibitor (as described above) to a translational activator. Specifically, NMDA receptor stimulation activates Aurora kinase, which phosphorylates CPEB, leading to the recruitment of cleavage and polyadenylation specificity factor (CPSF), which induces poly(A)-tail elongation, release of neuroguidin from eIF4E leading to translational activation [25]. This mechanism appears to promote translation of CamKIIα mRNA, along with a number of other mRNAs, following a variety of stimuli [26].

Studies of CamKIIα translation in dendrites of *Drosophila* neurons during long-term memory formation have revealed a role for activity-dependent relief of miRNA-mediated translational repression [62]. Here, cholinergic stimulation was found to degrade Armitage, a component of the RISC complex that is necessary for miRNA-mediated translational silencing. Degradation of Armitage led to increased translation of CamKIIα in dendrites, presumably by relieving miRNA inhibition. Intriguingly, Armitage was degraded by the ubiquitin proteasome pathway, providing a mechanistic link between the regulation of local protein synthesis and degradation during memory [63]. While these studies suggest that regulated degradation of RISC components can function to release miRNA translational inhibition, whether and how this could occur in a miRNA-specific manner remains unknown. More recent studies in rodent hippocampal neurons revealed that the mammalian homolog of Armitage, MOV10 also undergoes activity-dependent degradation via the ubiquitin-proteasome pathway [64] MOV10 degradation was triggered by NMDA receptor activation, and promoted the translation of a panel of dendritically localized mRNAs, including those encoding Limk1 and the depalmitoylating enzyme lysophospholipase1 (Lypla1), transcripts whose dendritic translation was previously shown to be regulated by miR134 and miR138 [41,64].

An additional mechanism whereby stimulation can activate translation is by selective translation of mRNAs containing Internal Ribosomal Entry Sites (IRESs). Translation of IRES-containing mRNAs is cap-independent and IRES trans-acting factors (ITAFs) traffic the ribosome to the start site, bypassing the need to scan from the 5'-UTR. Such transcripts may be preferentially translated when cap-dependent translation is inhibited. Of note, several dendritically localized transcripts seem to contain IRESs, although no studies have shown activity-dependent, IRES-mediated translation of these localized mRNAs [65]. In Aplysia neuroendocrine bag cells, however, Sossin and colleagues have shown that translation of the mRNA encoding egg-laying hormone (ELH), an mRNA that localizes to distal neurites, undergoes activity-dependent translational activation by switching from cap-dependent to IRES-dependent translation [66].

Another way to achieve specificity of translational regulation is to regulate the dendritic/synaptic localization of mRNAs. Several transcripts undergo activity-dependent transport into dendrites, including mRNAs encoding BDNF, TrkB, eIF4E and Arc [59,67,68]. Following stimulation, Arc mRNA is not only targeted into dendrites, but it also concentrates specifically at stimulated synapses [59]. The dendritic localization of BDNF mRNA has recently been shown to be regulated by alternative 3'-polyadenylation, such that only isoforms containing the long 3'-UTR are localized to dendrites [69]. A recent study of a sensorin translational reporter in *Aplysia* sensory-motor neurons found that serotonin only induced translation of the reporter when it localized to sensory-motor synapses [9], indicating that the particular subcellular localization of an mRNA within the neuronal process is critical to its translational regulation.

Can distinct stimuli regulate translation of specific localized transcripts?

The discovery of hundreds of dendritically localized mRNAs, and the elucidation of mechanisms for transcript-specific translational repression and activation, suggest that distinct stimuli might regulate the translation of distinct subsets of mRNAs. Consistent with this possibility, dynamic imaging of sensorin translation in *Aplysia* sensory neurons revealed translational induction during serotonin-induced long-term facilitation but not during FMRFamide-induced LTD of sensory-motor synapses [9]. In contrast, however, both LTP- and LTD-inducing stimuli stimulate translation of Arc [57,58,70] and EF1α [49,71] mRNAs in dendrites of rodent hippocampal neurons. The stimuli used to elicit LTP and LTD in these studies included bath application of BDNF and DHPG, as well as high

frequency tetanic stimulation. Understanding whether and how specific types and patterns of stimulation regulate translation of specific mRNAs may require monitoring translation following more physiologically relevant stimuli.

Dysregulation of local translation underlies human neurological disorders

Given the central function of local translation during the formation and function of neural circuits, it is not surprising that mutations in genes involved in local translation have been found to underlie several human neurological disorders. Of these, studies of Fragile X Mental Retardation have provided the most insight into both the physiological function of local translation and the pathophysiology of the disease.

Dysregulation of local translation in Fragile X

Fragile X syndrome is the most common heritable form of mental retardation and the most common known genetic cause of autism [44,72,73]. Fragile X syndrome is caused by loss-of-function mutations in FMRP, which is encoded by the *FMR1* gene located on the X chromosome. In humans, Fragile X syndrome typically results from expansion of a CGG repeat sequence in the 5'-untranslated region and silencing of the *FMR1* gene [74,75]. Patients with the Fragile X syndrome exhibit a wide-range of neurological deficits including cognitive impairment, seizures, emotional instability, sleep disorders, attention deficits, autonomic dysfunction, and autism [44,72,73]. FMRP, the gene product of the *FMR1* gene, is an RNA binding protein that associates with a large array of mRNAs, many of which encode proteins important for neuronal development and plasticity. In neurons, FMRP recruits CYFIP1 to target mRNAs, repressing their translation [24]. FMRP controls activity-dependent dendritic mRNA localization, stability and translational efficiency of dendritic mRNAs in response to stimulation of mGluRs [44,72,73].

FMRP is detected in cell bodies, in dendritic shafts and branch points [76], and at the base of synaptic spines and spine heads [77]. FMRP mRNA is translated near synapses in response to neurotransmitter activation [78]; moreover, different models of experience-dependent plasticity such as whisker stimulation [79,80], visual experience [81], and exposure to enriched environment [82] also promote FMRP translation. In addition, FMRP modulates the synaptic translation of other mRNAs in a neurotransmitter-dependent manner [58,83].

Mice lacking FMRP exhibit abnormalities in dendritic spine morphology, cognitive deficits exaggerated LTD, which is protein synthesis-independent at Schaffer collateral to CA1 pyramidal cell synapses of the hippocampus and decreased LTP in the cortex [44,72,73]. Insights into the molecular cascades that link overactivated group I mGluRs to exaggerated mGluR-LTD have recently been revealed. Recent findings indicate that in wild-type neurons, FMRP represses an array of mRNAs implicated in synaptic plasticity and spine morphogenesis including PI3K-enhancer (PIKE), a direct target of FMRP (Jennifer Darnell, personal communication). In hippocampal neurons of *Fmr1*-deficient mice, upregulation of PI3K-enhancer PIKE, an upstream regulator of PI3K/Akt signaling, results in overactivation of PI3K/Akt and mTOR signaling in the hippocampus, as assessed by several functional read-outs, including formation of the eIF4F translation initiation complex [84]. These findings are consistent with a model whereby in the Fragile X mouse, the protein/s required for mGluR-dependent AMPA receptor internalization and mGluR-LTD are already accumulated at Schaffer collateral to CA1 synapses under basal conditions [85]. These observations provide an important functional link between overactivated mGluR signaling, aberrant protein synthesis, and exaggerated mGluR-LTD in *Fmr1* KO mice [84]. Several neurotransmitter receptors are dysregulated in the absence of FMRP. For example, the *Fmr1* KO mouse exhibits aberrant numbers and/or activity of mGluR5 [86,87], dopamine [88] and AMPA [89] receptor signaling.

A hallmark feature of Fragile X syndrome in humans, also observed in the *Fmr1* KO mouse, is that of synaptic spine dysmorphogenesis [90]. Spines on hippocampal and cortical neurons of *Fmr1* KO mice exhibit enhanced density and are thinner and longer than those of age-matched wild-type mice, resembling an immature morphology [90–93]. These observations indicate that FMRP might normally repress local translation of proteins that inhibit synapse maturation, stabilization, and elimination [90]. On the other hand, a combination of imaging and electrophysiological studies indicate that in the absence of FMRP there is a delay in establishing synaptic connections [94] and a persistent, reduction in connectivity between neurons in the somatosensory, barrel cortex of Fragile X mice [95]. These observations raise the possibility that the increased number of spines on pyramidal cells might represent a compensatory mechanism to balance [or counter] the decrease in synapse connectivity. In the somatosensory cortex, although spine dysmorphogenesis persists into adulthood [92,93], it can be rescued by exposure of *Fmr1*-deficient mice to an enriched environment [92].

Conclusions

Over the past decade, the study of local translation in neurons has evolved from asking questions about whether and when local translation occurs to asking more mechanistic questions about how mRNA localization and translational regulation occur. In this review, we highlighted new insights into the mechanisms whereby mRNAs are transported in a translationally repressed state to specific subcellular compartments, focusing on repression of translation by eIF4E-BPs and related proteins, and on transcript-specific translational repression by miRNAs. We then considered mechanisms whereby distinct patterns of synaptic stimulation regulate translation of localized mRNAs. Here we focused on recent studies revealing central roles for the mTOR and S6K signaling pathways in regulating translation initiation at synapses, and on studies indicating a critical role for the miRNA pathway in local translation. Finally, we concentrated our attention on the many insights into the function and molecular mechanisms of local translation that have been garnered from studies of Fragile X Mental Retardation, a form of mental retardation caused by mutations in an RNA binding protein and translational repressor, FMRP. As we hope our review of the field indicates, new technologies that allow mRNA localization and regulated translation to be analyzed, manipulated and dynamically visualized in neurons are revealing complex, finely tuned functions for local translation in neuronal structure and function. As is often the case, these studies have given rise to a new set of questions, many of which are listed in Box 3. Challenges for the future include developing approaches to address these questions, and in particular developing technologies to study local translation in intact, complex neural circuits, thereby permitting investigation into the function of local translation in the nervous system of living animals.

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Box 1: RNA-binding proteins acquired in the nucleus affect the cytoplasmic fate of mRNAs

Many proteins that bind to mRNAs in the nucleus have been shown to remain bound in the cytoplasm and to regulate mRNA localization and translation. Such proteins include heterogeneous nuclear ribonucleoproteins (hnRNPs), exon junction complexes (EJC) and a number of other nuclear RNA binding proteins.

hnRNP A2 binds an RNA element, called the A2 response element (A2RE) within the 3'-untranslated region (UTR) of the mRNA encoding myelin basic protein (MBP) and localizes MBP mRNA to the distal processes of oligodendrocytes [96]. In neurons, A2REs mediate dendritic localization of reporter transcripts, and a subset of dendritically-localized mRNAs contain A2REs, indicating a role for hnRNP A2 in dendritic mRNA targeting [97].

The EJC consists of a set of nuclear proteins that bind to pre-mRNA transcripts during splicing and that have been shown to be required for the cytoplasmic localization of *oskar* mRNA in Drosophila oocytes [98]. Many EJC components are present in neuronal dendrites, where they have also been shown to bind localized mRNAs [99]. When EJC components are bound within coding regions, they may recruit ribosomes to promote translation; when they are deposited downstream of coding regions, the transcripts are usually targeted for degradation *via* the Nonsense-Mediated Decay (NMD) pathway after the first round of translation. The mRNA encoding Arc contains two conserved introns within its 3'-UTR and thus may be rapidly degraded by NMD after the first round of translation [100]. Such regulation may ensure tight temporal control and a 'burst' of Arc protein synthesis at stimulated synapses.

The zip-code binding proteins ZBP1 and ZBP2 associate with β-actin mRNA during transcription and are required for both export and dendritic targeting of β-actin mRNA [99].

ELAV/HuD proteins, neuron-specific nuclear RNA binding proteins known to regulate mRNA stability, have been shown to associate with many localized mRNAs, including those encoding GAP43, Homer 1a, neuritin and CamKIIα [101].

LSm1, an auxiliary factor for RNA degradation, and CBP80, a (pre)mRNA binding protein have been shown to bind to dendritically localized mRNAs β-actin, eEF1α, and the IP3 receptor [102].

Box 2: Heterogeneous populations of RNA granules in dendrites

mRNAs associate with distinct sets of proteins throughout their life cycle, from transcription to degradation, through a process of ribonucleoprotein (RNP) remodeling. The population of dendritic mRNPs is thus heterogeneous and dynamic, reflecting both the diversity of localized mRNAs and the transition between states of translational repression, activation and degradation. Understanding the composition of, and the relationship between, various dendritic RNA granules is a focus of research in the field.

RNA Transport Granules. Biochemical fractionation reveals that dendritically localized mRNAs are present in small RNPs, free of ribosomes, as well as in larger structures that contain ribosomes, kinesin and the RNA binding protein Staufen [103]. Affinity purification for kinesin interacting proteins in brain led to the identification of larger (~1000S) RNA transport granules containing localized mRNAs and several dozen proteins, including translation factors and repressors [104]. In a separate study, Elvira et al. [105] purified and characterized large RNA transport granules composed of a distinct set of localized mRNAs and proteins, indicating that distinct mRNAs are transported in distinct RNA granules.

Stress Granules (SGs) temporarily arrest mRNA translation when cells are stressed (e.g. during oxidative or metabolic stress). Once the stress is relieved, SGs dismantle and mRNA can resume normal activity [106]. SGs have been detected in dendrites, where they may form dynamically by recruitment of Pumilio proteins to RNAs and subsequent aggregation [33].

Processing Bodies (p-bodies) function to prime and process mRNAs for degradation and contain decapping enzymes, exonucleases, miRNAs and components of the RISC machinery. Many p-body markers have been detected in neuronal dendrites [107,108]. A distinct class of p-bodies, termed dendritic p-body like structures, or dlPBs, have been detected in hypothalamic and hippocampal neurons that contain many components of p-bodies but lack the exonuclease Xrm1 [109], and may thus serve as storage sites for translationally repressed mRNAs.

Box 3: Outstanding questions in the field of local translation at the synapse

- What is the nature of the cis-acting RNA localization elements that target transcripts to specific subcellular compartments within a neuron?
- What are the RNA binding proteins that function to localize mRNAs within neurons and how do they mediate this localization?
- What is the composition of the RNPs that localize mRNAs to dendrites? What is the relationship between RNA transport granules, P-bodies and stress granules?
- What are the physiologically relevant stimuli that regulate local translation?
- Do/how do distinct stimuli regulate the translation of specific subsets of transcripts?
- How does the miRNA pathway contribute to local translation at synapses and thus synaptic plasticity?
- Are there differences in the mechanisms of translational regulation at the synapse as compared to in the soma?
- What mechanisms at synapses facilitate folding and maturation of the newly synthesized proteins?
- What is the nature of the secretory pathway, which is necessary for synthesis of membrane and secreted proteins, in distal dendrites?
- How does local translation of specific transcripts contribute to or alter the function of neural circuits?
- Is/how is stimulus-induced transcriptional regulation in the nucleus integrated with stimulus-induced local translation at the synapse?
- What are the proteins critical to mGluR-LTD that are accumulated at CA1 synapses of Fragile X mice?
- Is PI3 kinase enhancer (PIKE) an FMRP target critical to aberrant protein-synthesis dependent synaptic plasticity observed in Fragile X mice?

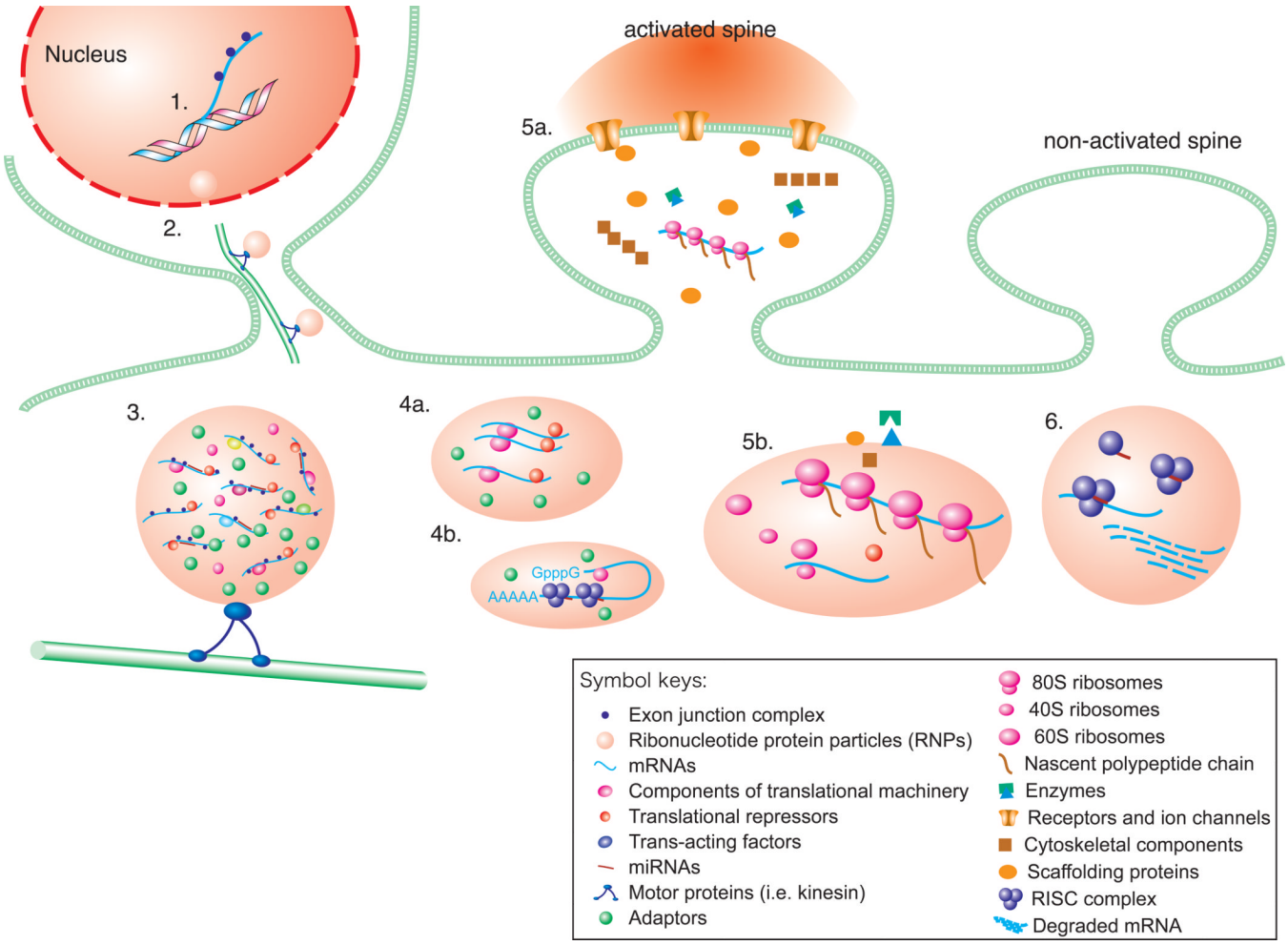

Fig. 1. Model of mRNA transport and regulated local translation in dendrites

1. Transcription of mRNAs and co-transcriptional recruitment of transacting factors such as the exon junction complex; 2. Assembly and export of ribonucleotide protein particles (RNPs); 3. The cytoplasmic RNP is packaged into a transport granule consisting of mRNAs; components of the translational machinery, translational repressors; trans-acting factors; miRNAs, kinesin and adaptors, which are transported along microtubules by molecular motors into dendrites; 4. Mechanisms by which dendritic mRNA are thought to be maintained in a translationally repressed state are a) repression by assembly of mRNAs together with transational repressors into structures known as RNPs; and b) repression by assembly of mRNAs together with miRNAs to form the RISC complex within structures known as process-bodies (P-bodies); 5. a) Activation of neurotransmitter receptors and voltage-gated ion channels engages intracellular second messenger cascades such as the mTOR pathway, which promote translation by turning on the translational machinery and/or by removing translational repressors; b) Active translation at a stimulated synapse 80S ribosome, 40S ribosomes, 60S ribosomes, nascent polypeptide chain; newly synthesized proteins (enzymes, receptors and ion channels, cytoskeletal components, scaffolding proteins) are incorporated into the synapse; 6. Local degradation of mRNAs. miRNA-RISC-mediated local degradation of mRNAs in P-bodies (RISC complex; mRNA prior to degradation; degraded mRNA; miRNA).

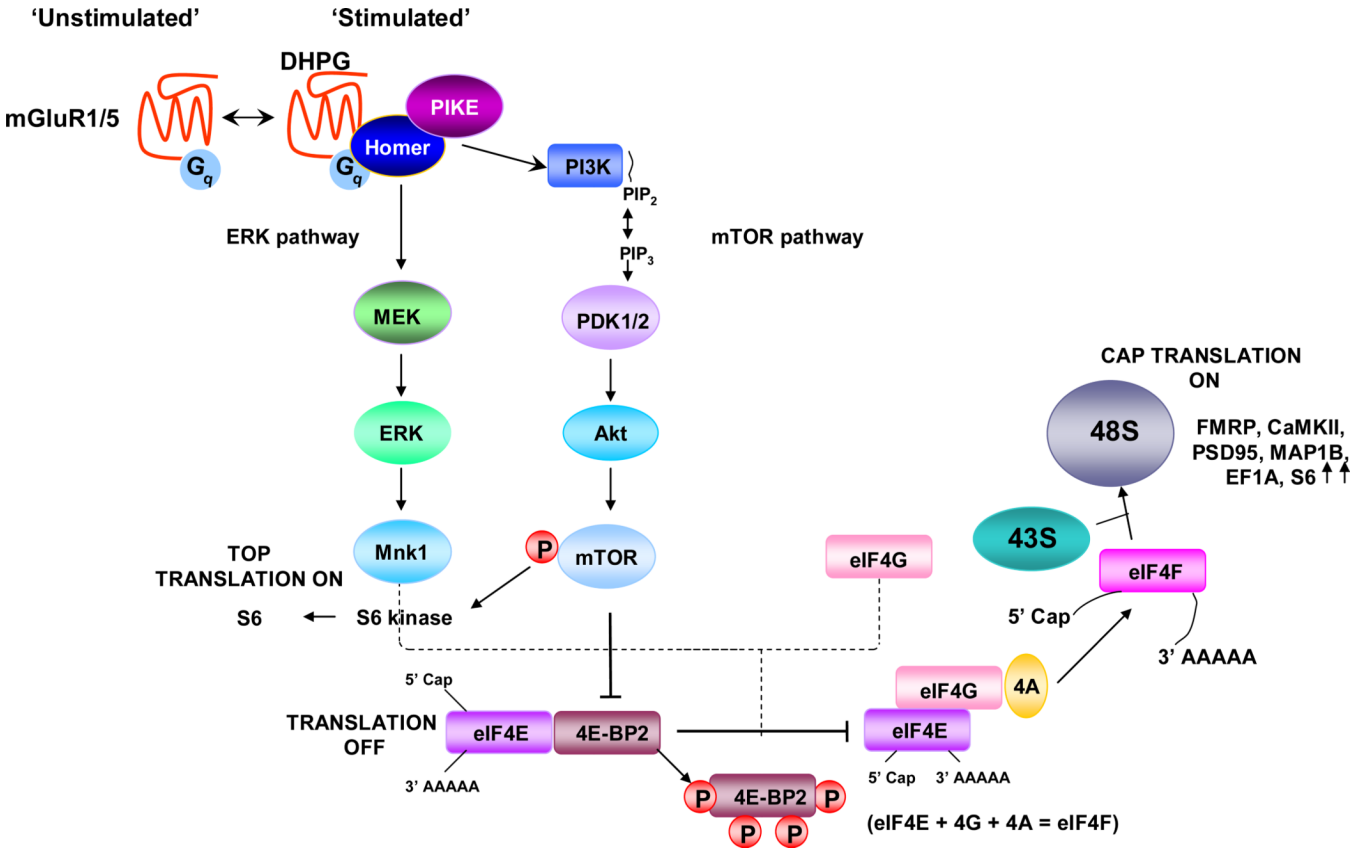

Fig. 2. Signaling pathways that regulate mTOR-dependent local protein synthesis in dendrites

Activation of group I metabotropic glutamate receptors (mGluRs) engages the ERK/MAPK and phosphatidylinositol 3-kinase (PI3K) signaling pathways. PI3K acts *via* PDK1/2 to phosphorylate and activate Akt. Akt phosphorylates and activates the mammalian target of rapamycin (mTOR). Upon activation, mTOR phosphorylates and activates S6 kinase 1 (S6K1). Phosphorylation (P) of eIF4E-binding protein (4E-BP) by mTOR promotes the release of 4E-BP from the eukaryotic translation initiation factor 4E (eIF4E), enabling eIF4E to assembly with eIF4G and form the active translation initiation complex eIF4F, composed of eIF4E, eIF4A and eIF4G. eIF4F recruits the mRNA to the 43S pre-initiation complex to form the 48S initiation complex. The eIF4F complex and the poly(A) tail act synergistically to stimulate mRNA translation. ERK phosphorylates and activates MAPK-interacting serine/threonine kinase 1 (Mnk1), which phosphorylates eIF4E. S6K1 phosphorylates and activates ribosomal protein S6, stimulating TOP-dependent translation. The intracellular signaling pathways depicted here are also activated by brain-derived neurotrophic factor (BDNF) in the hippocampus and cultured cortical neurons, and by serotonin in *Aplysia californica* sensory neurons. m7G, 7-methyl-GTP; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-trisphosphate. Adapted from [46].

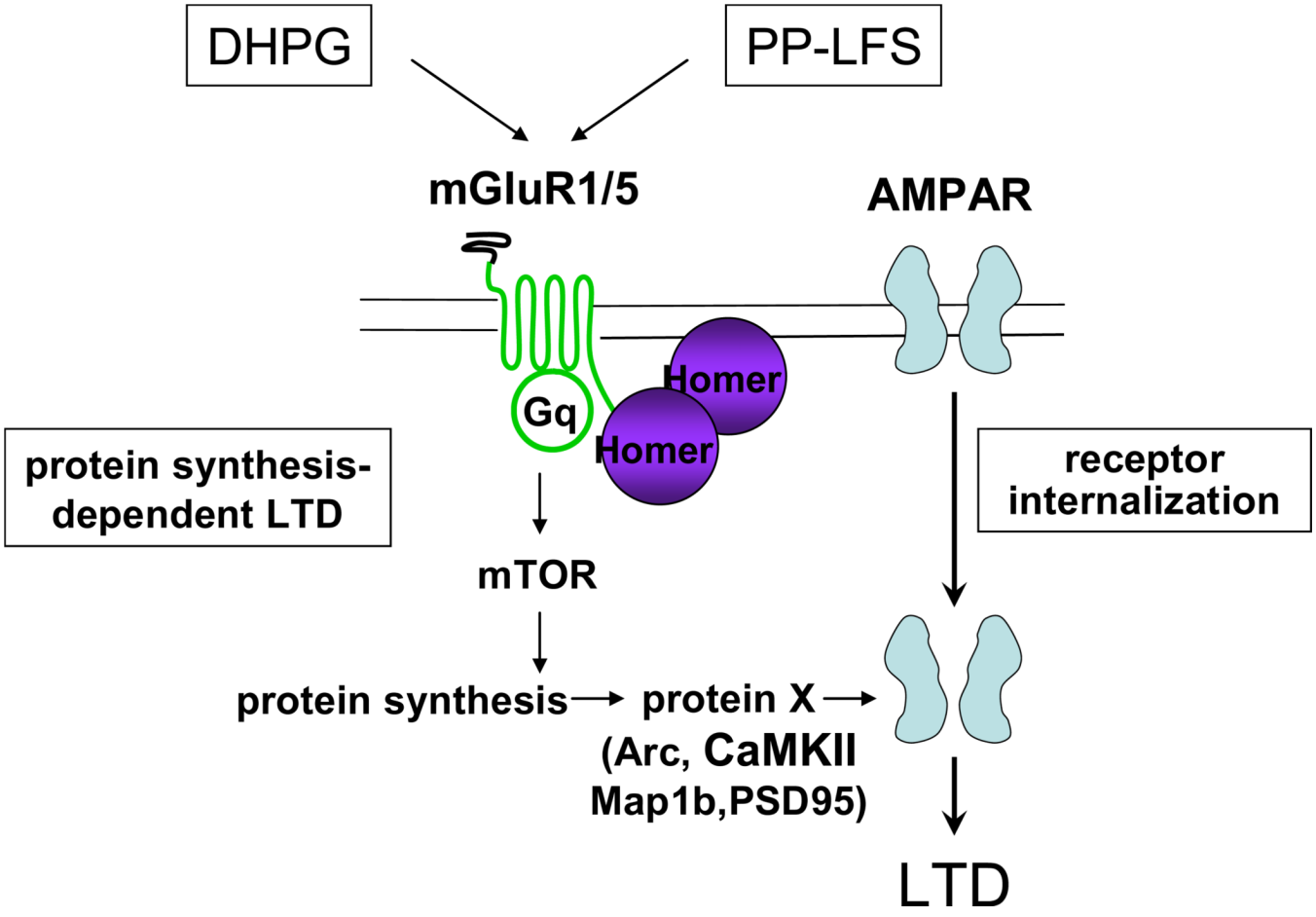

Fig. 3. mGluR-LTD at CA1 synapses of adolescent wild-type mice requires local protein synthesis

Induction of mGluR-LTD by either bath application of the group I mGluR agonist DHPG or by paired pulse low frequency stimulation (PP-LFS) promotes the internalization of synaptic AMPARs in a group I mGluR-dependent manner, leading to a decrease in synaptic efficacy or LTD. mGluR-dependent internalization of AMPARs at CA1 synapses of adolescent wild-type mice requires mTOR-dependent local protein synthesis of an as yet unknown protein (*eg.*, Arc, MAP1b, PSD-95, CaMKII). Correct coupling of Homer to mGluR1/5 is required for activation of mTOR, AMPAR internalization and mGluR-LTD [15, 42]. Courtesy of Huber KM.