Mechanisms of translational regulation in synaptic plasticity

Wayne S Sossin¹ and Jean-Claude Lacaille²

¹ Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec H3A 2B4, Canada

² Département de physiologie, GRSNC, Université de Montréal, C.P. 6128, succ. Centre-ville, Montréal, Québec H3C 3J7, Canada

Abstract

The plasticity of the nervous system is due to the ability of neurons to change their properties by altering the function of their proteome. A major mechanism for this is through altering the amount of proteins by regulating their translation. In this review, we focus on recent advances in the elucidation of the mechanisms by which neurons regulate translation during synaptic plasticity. Particular focus will be on the different transduction mechanisms that selectively target distinct elements of the mRNA in the regulation of translation during plasticity.

There have been a number of recent reviews on the role of translation in regulating synaptic plasticity, either on local translation, transport of mRNAs or in particular on the target of rapamycin complex 1 (TORC1) system [1–4]. Translation is important for many aspects of synaptic plasticity: 1) altered translation even before stimulation may change the proteome of the neuron and thus its ability to undergo plasticity, 2) the plasticity-inducing stimulus may increase or decrease the translation of prelocalized mRNAs to affect synaptic strength, 3) translation can be important in controlling the transcription required for long-term changes, and 4) translation of newly transcribed mRNAs will also be highly regulated in plasticity. Here we describe new advances in the role of distinct elements of the mRNA in mediating regulation by multiple signalling pathways, emphasizing the mRNA as a key control point in translational control of synaptic plasticity (Figure 1).

The cap: eIF4E and 4E-BPs

At the N-terminal end of the mRNA is a 7-methyl GTP cap (Figure 1). The eukaryotic initiation factor (eIF) 4F complex consisting of eIF4A, eIF4B, eIF4E, and eIF4G associates to bind the cap-structure and this step is important for bringing the mRNA to the ribosome. A rate-limiting factor is the availability of eIF4E, the component of the eIF4F complex that directly recognizes the cap. Under basal conditions, translation is repressed as eIF4E is sequestered by binding proteins (eIF4E binding proteins, 4E-BPs).

Evidence using transgenic approaches point to repression of translation by 4E-BPs as important in regulating synaptic plasticity. First, mice with a knockout of 4E-BP2, the major

4E-BP isoform present the brain, show facilitation of late-long-term potentiation (L-LTP) induction in hippocampal pyramidal cells [5]. A single 100 Hz train that produces only early-LTP in normal animals, produces L-LTP in 4E-BP2 knock out (KO) mice. Similar results are seen in metabotropic glutamate receptor (mGluR)-induced late-LTP in interneurons, where a single stimulation of mGluR1, which does not elicit late-LTP in normal mice, does elicit late-LTP in 4E-BP1/2 KO mice [6]. Moreover, 4E-BP2 KO mice also show enhancement of translation-dependent mGluR-long-term depression (LTD) in pyramidal cells [7]. Thus, proteins that are rate-limiting for inducing synaptic plasticity in both directions are normally repressed by 4E-BP.

TORC1 is an important regulator of translation (see Box 1) and TORC1 dependent events can be probed using the specific inhibitor rapamycin. An important TORC1 target is 4E-BP, whose repression of eIF4E is removed by rapamycin-dependent phosphorylation. Since rapamycin blocks the same forms of plasticity effected by 4E-BP2 knockdowns (late-LTP in CA1 pyramids and interneurons, and mGluR-LTD) [1], the implication has been that the role of TORC1 in these forms of plasticity is through phosphorylation of 4E-BP2. However, in mature brain, regulation of 4E-BPs may involve additional mechanisms other than phosphorylation of 4E-BPs [8*]. There is little if any 4E-BP phosphorylation after stimulation in mature brain, but instead 4E-BP2 undergoes de-amidation resulting in enhanced association with the TORC1 component Raptor (Box 1), reduced binding to eIF4E and thus reduced inhibition of 4E-BP targets. Using a different approach, overexpressing a form of 4E-BP that cannot be regulated by TORC1 and thus does not allow TORC1 mediated removal of 4E-BP repression, regulation of 4E-BP was found to be dispensable for TORC1-dependent long-term plasticity in *Aplysia* [9]. Thus, while proteins important for plasticity are clearly regulated by 4E-BP, it is still unclear if the target of TORC1 in inducing synaptic plasticity is 4E-BPs.

Box 1

TORC1 pathway

The protein kinase TOR is a component of multiple protein complexes with distinct roles. TORC1 is defined by its sensitivity to rapamycin and contains the distinct protein component Raptor. Raptor plays an important role in bringing substrates to TORC1. TORC1 is also specific in its activation by the small G protein Ras homolog enriched in brain (Rheb). Rheb is regulated by the GTPase activating protein (GAP) activity of the tubular sclerosis complex (TSC)-1, TSC-2 complex and growth factors activate TORC1 through inactivation of this GAP [60]. TORC1 is sensitive to amino acids through the Ras related GTPases (Rag) family that also interact with Raptor [60]. Finally, energy levels communicate to the TORC1 complex through AMP kinase, whose activation under low ATP levels inactivates TORC1 through direct phosphorylation or both TSC-2 and Raptor.

Evidence from other systems suggests 4E-BP-dependent regulation of specific mRNAs with highly structured 5' untranslated regions (UTR)s (Figure 1). In the immune system, there is a 4E-BP1/2-dependent regulation of innate immune response specifically via interferon regulatory factor 7 (Irf7) messenger RNA translation [10*]. The identity of specific mRNAs

regulated in similar fashion in synaptic plasticity is a crucial issue that remains to be explored. Protein kinase M(PKM) ζ , a constitutively active form of protein kinase C (PKC), is a key kinase that is locally translated, contains a highly structured 5' UTR and is required for the maintenance of LTP and long-term memory by increasing the levels of AMPA receptors [11,12]. Recently, inhibitors of TORC1 signalling pathways were found to prevent synthesis of PKM ζ [13]. It will be interesting to determine if this is through 4E-BP regulation.

TORC1 and cap-dependent translation are also important for synaptic plasticity in other systems. Notably, in ventral tegmental neurons, activation of mGluRs leads to long-term depression through the rapid synthesis of lower conductance GluA2 subunits via TORC1 and their synaptic insertion [14]. TORC1 translational control is thus crucial for the reversal of cocaine-induced strengthening of excitatory synapses in dopaminergic neurons, and relevant to addiction.

It should also be noted that increased activation of the TORC1 system is detrimental to synaptic plasticity. Hyperactive TORC1 signalling is associated with deficits in cognitive functions [5,15^{**},16,17], which in some cases can be rescued by rapamycin [15^{**}]. The proteins synthesized under these conditions that block plasticity have not been identified.

Although TORC1 is a crucial regulator of cap-dependent translation, other TORC1 independent mechanisms also control eIF4F and synaptic plasticity. At perforant path synapses of dentate gyrus granule cells, late-LTP and activity-regulated cytoskeleton-associated protein (Arc) protein synthesis are not blocked by rapamycin, but require increased eIF4F formation downstream of Mnk-signalling, [18^{*}]. Mnk is the eIF4E kinase and may regulate eIF4F formation through eIF4E phosphorylation, although how eIF4E phosphorylation regulates translation is a highly debated question [19].

Elements in the 5' UTR: oligopyrimidine tracts and upstream open reading frames

Another downstream pathway of TORC1 involve selective upregulation of 5' terminal oligopyrimidine (5' TOP) mRNAs that encode components of the translational machinery (Figure 1). High frequency stimulation induces late-LTP and increased translation of 5' TOP containing mRNAs via convergent PI3K and ERK stimulation of TORC1 [20]. Recently, a 5' TOP-myr-dYFP reporter was generated to selectively monitor 5' TOP translation in pyramidal neurons in slice cultures [21]. Forskolin, an adenylate cyclase activator, induced late-LTP and upregulated dendritic translation of the 5' TOP mRNA reporter via TORC1, suggesting that upregulation of the translational machinery is a candidate mechanism in late-LTP. Interestingly, while TOR is important for this regulation, raptor is not, suggesting that some TORC1 functions do not depend on raptor [22^{*}].

After the ribosome is recruited to the 5' cap at the start of an mRNA, scanning proceeds to look for an open reading frame (ORF). Upstream ORFs (uORFs) (Figure 1) that are out of frame from the coding region initiate translation, and thus reduce translation of the coding region. uORFs allow regulation at the level of eIF2 α phosphorylation (Box 2). Interestingly,

one such regulated mRNA is the transcriptional regulator activating transcription factor 4 (ATF4), also known as the cyclic-AMP response element binding protein (CREB) repressor. Decreases in eIF2 α phosphorylation that occur during the induction of LTP lead to decreases in translation of ATF4 and consequent upregulation of the transcription of CREB-regulated genes important for the induction of late-LTP. Thus, genetic reduction in eIF2 α phosphorylation leads to enhanced induction of late-LTP and learning [23]. This result has recently been confirmed using a transgenic mouse where eIF2 α phosphorylation could be increased through drug application [24]. The increased eIF2 α phosphorylation led to increased ATF4 synthesis with decreased LTP and memory, the converse result from that seen when decreasing eIF2 α phosphorylation. Interestingly, these results were seen in the absence of changes in overall protein synthesis, suggesting that it is the specific regulation of proteins like ATF4 and not changes in overall translation that are important for the regulation of plasticity.

Box 2

eIF2 α phosphorylation

Phosphorylation of eIF2 α generates a dominant negative form of the protein that reduces levels of GTP-loaded eIF2-Formyl-Met tRNA required for loading of the initiating methionine [61]. Phosphorylation of eIF2 α is mediated by four dedicated kinases, GCN2, PERK, HIF-1, and PKR [61]. When the GTP-loaded eIF2-Formyl-Met tRNA levels are low owing to eIF2 α phosphorylation, overall translation initiation is slowed, but in addition upstream ORFs with methionines that are in a suboptimal context can be read through and mRNAs that are regulated in this fashion are upregulated [61].

Coding region: elongation and eEF2 phosphorylation

While translation initiation has normally been thought to be the regulated rate-limiting step in translation, recent results suggest regulation of elongation is also important for the translational regulation of synaptic plasticity. Elongation is inhibited by phosphorylation of eukaryotic elongation factor 2 (eEF2), a factor important in the elongation phase of translation (Box 3). Calcium entry through NMDA receptors during spontaneous synaptic transmission activates eEF2 kinase, thus tonically inhibiting local translation [25]. Blocking these miniature events decreases eEF2 phosphorylation and increases translation locally leading to homeostatic increases in synaptic strength. By contrast, activation of eEF2 kinase through mGluRs during LTD, while decreasing overall translation, activates translation of Arc, calcium-calmodulin kinase (CaMK)II, and microtubule associated protein 1B mRNAs and is crucial for translation-dependent mGluR-LTD through promotion of AMPA receptor endocytosis [26,27**]. In this case, it is hypothesized that the stalling of elongation by phosphorylation of eEF2 releases a rate-limiting factor that allows increased translation of these mRNAs. Indeed, stalling elongation using low concentrations of cycloheximide increases translation of Arc. Also low concentrations of cycloheximide can rescue LTD when eEF2 kinase is removed, demonstrating that the requirement for eEF2 kinase during mGluR-LTD is to stall translational elongation [27**]. These transcripts are also regulated by fragile X mental retardation protein (FMRP), but this appears to be distinct, in that removal

of FMRP leads to premature translation of these messages and protein-synthesis independent mGluR-LTD [3], while removal of eEF2 kinase prevents translation of these mRNAs and leads to a loss of mGluR-LTD [26,27**].

Box 3

eEF2 phosphorylation

eEF2 promotes the GTP-dependent translocation of the nascent protein chain from the A-site to the P-site of the ribosome. It is inactivated by phosphorylation by a dedicated kinase, eEF2 kinase. This kinase is activated by calcium-calmodulin and was initially known as CAMKIII. The kinase is highly regulated by phosphorylation, and in particular is inactivated by rapamycin-sensitive phosphorylation at both S6 kinase dependent and independent sites [62]. The ability of the kinase to be activated by calcium and inactivated by TORC1 allows it to be bidirectionally regulated during plasticity [27**, 28,18*,29]. Indeed, in hippocampal cultures, while NMDA receptors activated by minis led to increases in eEF2 phosphorylation, action potentials led to decreases in eEF2 phosphorylation suggesting a sensitive balance between these two regulatory mechanisms [25].

eEF2 phosphorylation has been shown to be regulated by a number of paradigms of synaptic plasticity, but in different directions. As described above mGluR-LTD leads to an increase in eEF2 phosphorylation [27**] as does taste conditioning [28] and LTP induction in the dentate gyrus [18*]. While eEF2 phosphorylation is important for mGluR-LTD, it is not required for NMDA dependent LTD. By contrast, fear conditioning leads to a decrease in eEF2 phosphorylation [29]. Also eEF2 kinase knockout animals have enhanced CA1 late-LTP [27**] and increasing eEF2 phosphorylation by overexpression of eEF2 kinase blocks late LTP in the hippocampus and long-term memory [29].

It is interesting that loss of FMRP lead to memory deficits in *Drosophila* [30] and similar to mammalian systems this appears to be due to increased synaptic outgrowth downstream of excess mGLUR signalling [31]. Fragile X in humans, the disease caused by the loss of this protein, also shows cognitive deficits and morphological abnormalities [32]. In *Drosophila*, this has recently been linked directly to increased protein synthesis and protein-synthesis inhibitors can rescue the loss of FMRP in *Drosophila* [30]. However, eEF2 kinase regulation is not important in *Drosophila*, since this kinase is not present in insects, although it is present in most other invertebrates.

Stop codon and nonsense mediated decay

When the ribosome reaches the stop codon, an additional cohort of proteins is recruited to release the ribosome from the mRNA. A crucial one is the RNA helicase, up frame shift 1 (Upf1) (Figure 1). An important role of Upf1 is to signal degradation of mRNAs with inappropriate stop codons (usually due to inaccurate splicing in the nucleus) that are recognized through interactions between Upf1 and proteins at exon junction complexes (EJCs) located downstream of the stop codon. Interestingly, eIF4AIII, a component of the EJCs, was found associated with neuronal mRNA granules and dendritic mRNAs [33].

Moreover, eIF4AIII knockdown increased Arc mRNA and protein expression, as well as synaptic strength and GluR1 abundance at hippocampal synapses. In particular Arc mRNA, due to an intron in its 3'UTR, is naturally regulated through this mechanism [33].

Staufen is another RNA binding protein, which is present in dendritic mRNA granules and is involved in mRNA transport and translational regulation in synaptic plasticity. One isoform of Staufen, Stau 1, has also been shown to interact with Upf1 [34]. Knockdown of Stau 1 in hippocampal slice cultures, impaired translation-dependent late-LTP induced by forskolin, while leaving intact translation-independent early-LTP and mGluRLTD[35*]. However, mutant mice expressing a truncated Stau 1 protein lacking a functional RNA-binding domain 3, while showing deficits in dendritic delivery of β -actin mRNA-containing ribonucleoprotein particles and a significantly reduced dendritic tree, still showed normal hippocampus-dependent learning [36]. This may reflect a difference between the assays, or indicate that direct mRNA binding by Stau 1 is not required for its role in late-LTP. Staufen is also required for plasticity in *Drosophila* and *Aplysia* [37,38].

3'UTR: regulation by miRNAs

miRNAs target mRNAs mainly through binding to the 3'UTR and when miRNAs are bound, the Argonaute proteins are recruited to them RNA and repress translation (Figure 1). The exact mechanism employed by Argonaute is not clear, but recent evidence suggests that it requires binding of glycine tryptophan protein of 182 kDa(GW182) [39] and probably involves both repression of cap binding and deadenylation [40,41]. The first indication that miRNAs were involved in synaptic plasticity came from *Drosophila*, where regulated disruption of the silencing complex component Armitage, led to the removal of miRNA-mediated repression of CaMKII. This regulated increase in CaMKII synthesis occurred at synapses during memory formation [42]. Recently a similar regulation, degradation of the mammalian ortholog of Armitage, Moloney leukemia virus 10 homolog (MOV10), was discovered at mammalian synapses, linked to activity dependent removal of miRNA repression mediated by miR-138 and increased translation of CaMKII and a depalmitoylation enzyme, acyl protein thioesterase (APT)1 [43**]. Another group showed that miR-138 was enriched at synapses and controlled spine size through regulating levels of APT1 and could be rescued by expression of Gα13 a known target of this enzyme [44]. This suggest that activity-dependent changes in spine size, a process known to require translation activation for its stabilization [45], could be due to activity dependent synthesis of APT-1, followed by depalmitoylation of Gα13. Specific miRNAs have also recently been shown to be downregulated during long-term facilitation in *Aplysia*. In this case, a link has been made to upregulation of the CREB transcription factor involved in long-term memory formation [46], although the mechanism underlying the downregulation was not identified; it will be interesting to determine if it involves degradation of the RNA helicase (MOV10/armitage).

Poly A tail region and CPEB

The end of the mRNA has a poly A tail that is initially added in the nucleus and is modulated by cytoplasmic poly A polymerases and adenylases. The poly-A tail modulates translation through the binding of the poly-A binding protein (PABP) that in turn binds

eIF4G and, similar to eIF4E, assists in recruitment of the mRNA to ribosomes. A major regulator of this process is the cytoplasmic polyadenylation element (CPE) binding protein (CPEB) (Figure 1). Interestingly CPEB has been implicated in both translational repression through its interaction with eIF4E binding proteins, such as maskin and neuroguidin, and translational activation through stimulating increases in poly A tail length [2]. The repressing or activating role of CPEB may be regulated by the cobinding of another RNA binding protein Pumilio [47]. Transgenic mice with deletion of CPEB-1 show selective dysregulation of certain forms of hippocampal synaptic plasticity: theta burst stimulation-induced late-LTP, capture of late-LTP, and NMDAR-mediated LTD[48]. One important target of CPEB is translation of cJUN-1 [49].

Regulation of translation via CPEB is also involved in activity-dependent development and synaptic plasticity in other systems. In the optic tectum, synaptic activity leads to CPEB-1 phosphorylation, translational derepression of target mRNAs, and protein-synthesis enabling experience-dependent synaptic plasticity in developing visual system of *Xenopus laevis* [50]. In *Aplysia*, sustained CPEB-dependent local protein synthesis is necessary for the stabilization of newly formed synapses and the persistence of long-term facilitation and memory [51*]. In this case aggregation of CPEB to form prion-like multimers appears to be important for the persistence of this synaptic change [52]. Moreover, deleting the polyglutamine rich region of Orb2, a CPEB homolog expressed specifically in the brain of *Drosophila*, resulted in defective formation of long-term courtship memory [53] and Orb2, like *Aplysia* CPEB can multimerize [52]. While the relationship between the requirement for CPEB and polyadenylation activity has not been directly shown, the fact that a dominant negative form of the Gld2 poly(A) polymerase, the polymerase involved in CPEB-dependent polyadenylation, blocked olfactory long-term memory in *Drosophila* [54] is consistent with an important role for polyadenylation in synaptic plasticity.

5'-3'UTR interactions

In many cases translational repressors that bind to the 3'UTR work by limiting access to the cap. For example FMRP can work in this manner through binding to the adaptor protein cytoplasmic FMRP interacting protein (CYFIP), which then binds to the cap binding protein eIF4E [55] (Figure 1). Regulated disruption of this binding would then lead to increased access of eIF4G to eIF4E and translation initiation. Dialog between the 5' and 3'UTRs can also depend on specific sequences in the 5'UTR. A recent example of this is the local translational control of the sensorin mRNA in *Aplysia* sensory neurons. Local translation of sensorin is important for stabilization of nascent synapses and sensorin is required for long-term facilitation at this synapse [56,57]. While the 3'UTR is sufficient for transport of this message to processes, the 5'UTR is required for specific location to synapses. Using a fluorescent protein reporter hooked up to the 5' and 3'UTR it was shown that both of these sequences were required for local translation activation by 5-HT during long-term facilitation [58**].

Conclusions

One important lesson learned from recent results is that a multiplicity of mechanisms involving distinct elements of mRNAs evolved to control translation in synaptic plasticity. Even transduction mechanisms that would apparently only regulate overall translation, such as regulation of elongation, have specific effects on some mRNAs. There has clearly been extensive evolutionary pressure to enable specific transcripts to respond to a variety of different pathways. For example, while translation of pre-localized Arc message is important for mGluR-LTD [27^{**}], translation of newly transcribed Arc mRNA through Mnk activation is important for LTP in the dentate gyrus [18^{*}]. In addition Arc mRNA is regulated by the EJC [33]. In this case, the ability of a specific mRNA to be translationally regulated by distinct transduction mechanisms associated with different forms of plasticity is owing to multiple responding elements in their mRNAs allowing specific regulation by separate pathways. Interestingly, for Arc, differential levels of the protein are also associated with different forms of plasticity. Moderate increases in Arc are linked to mGluR-LTD, while large increases in Arc are associated with L-LTP [59] and thus the type of translational regulation may also be important for the type of plasticity generated.

All these recent findings point to the mRNA as a key control point in translational regulation of synaptic plasticity. Moreover, these mechanisms will also have to be integrated with mRNA elements important for its transport. Elucidating the mRNA sequences that optimize response to different signals and their mechanisms of interaction with each other will allow identification of the transform between signal transduction and the changes in the proteome underlying synaptic plasticity.

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Figure 1.

Key regulatory elements of mRNAs for translational control in synaptic plasticity. Elements of the mRNA are either red ovals (Cap, Stop, AUG), green boxes (sequence specific), or purple boxes (coding, poly A region). Proteins that interact with each element are indicated in relation with the appropriate region. The cap allows regulation through eIF4E and 4E-BP interactions, which are in turn regulated by TORC1 phosphorylation of 4E-BP and this interaction also regulates structured regions in the 5'UTR. Upstream ORFs allow positive regulation through eIF2 α since these uORFs are only read through when levels of eIF2 α are low. Dephosphorylation of eIF2 α also upregulates translation through increased recognition of the start codon. Elongation is regulated by calcium-activated eEF2-kinase phosphorylation of eEF2. This is negatively regulated by TORC1. The stop codon regulates through nonsense and Staufen mediated decay processes. The 3'UTR contains binding sites for miRNAs for translational regulation through Argonaute and repression is relieved through degradation of Armitage/MOV10. A number of RNA binding proteins have been implicated in plasticity. FMRP also regulates eIF4E through CYFIP. The CPE binds to CPEB that regulates both the length of the poly A tail and through Neuroguidin and Maskin, eIF4E. Pumilio regulates the actions of CPEB. The poly A tail stimulates translation through PABP and this in turn is regulated by CPEB-dependent polyadenylation.