Translational Regulatory Mechanisms in Synaptic Plasticity and Memory Storage

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Synaptic activity-dependent protein synthesis is required to convert a labile short-term memory (STM) into a persistent long-term memory (LTM). Indeed, genetic or pharmacological inhibition of translation impairs LTM, but not STM. Long-lasting biochemical and morphological changes of synapses, which underlie learning and memory, also require new protein synthesis. In recent years, a large number of experiments have yielded much new information about the processes that govern translational control of synaptic plasticity during learning and memory processes. Signaling pathways that modulate mRNA translation play critical roles in these processes. In this chapter, we review the mechanisms by which certain translational regulators including eIF2α, 4E-BP, S6K, and CPEB control long-term

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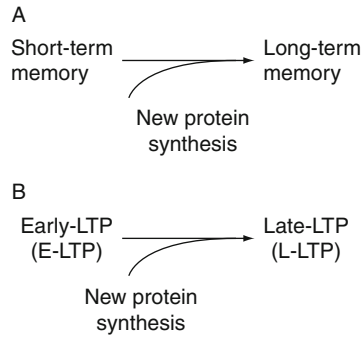

FIG. 1. Long-lasting neuronal processes require new protein synthesis. Both long-term memory (A) and long-lasting changes in synaptic strength (L-LTP) (B) depend on new protein synthesis.

synaptic plasticity and memory consolidation and their involvement in neurologic disease.

Two kinds of memory can be empirically distinguished: short-term memory (STM), which lasts between 1 and 3 h, and long-term memory (LTM), which could last for months and even many decades.[1,2] LTM, but not STM requires new gene expression which includes transcription and translation (Fig. 1). Memory arises through the connection of brain cells (neurons) and activity-dependent changes in synaptic strength are thought to modulate learning behavior.[3–5] To measure changes in synaptic strength, neuroscientists and physiologists use mainly two very well-characterized models, termed long-term potentiation (LTP) and long-term depression (LTD).[6] LTP and memory share similar molecular and cellular mechanisms. Thus, LTP is thought to be the main cellular model underlying learning and memory.[7,8] Like memory, LTP exhibits two distinct phases: a transient early LTP (E-LTP) depends on modification of extant proteins, whereas late LTP (L-LTP) requires transcription and new protein synthesis (Fig. 1). E-LTP is typically induced by a single train of high-frequency (tetanic) stimulation of an afferent pathway and lasts only 1–2 h. In contrast, L-LTP is generally induced by several repetitions of such stimulations (typically four tetanic trains separated by 5–10 min) and persists for many hours.[9,10]

I. Synaptic Plasticity Control by eIF2

Initiation is the rate-limiting step of translation and under most circumstances is the primary target for regulation, which is often mediated by reversible phosphorylation of initiation factors (see the chapters by Fraser and by Blenis, this volume). Two main mechanisms by which translation is controlled are the formation of the ternary complex via eIF2 and the recruitment of the ribosome to the mRNA via 4E-BPs.

As described in the chapter by Fraser, this volume, phosphorylation of an α subunit of eIF2 (at Ser51) converts the protein from a substrate to a competitive inhibitor of the GDP/GTP-exchange reaction by decreasing the rate of dissociation of eIF2 from eIF2B.[11,12] This causes a decrease in general translation initiation.[12,13] In mammals, the phosphorylation of eIF2α is a highly dynamic, regulated process that is controlled by a family of eIF2α kinases and two phosphatase complexes, for which eIF2α is their only known substrate.[14,15] This family of kinases includes the hemin-regulated inhibitor kinase (HRI), the double stranded (ds) RNA-activated protein kinase (PKR), the PKR-endoplasmic reticulum (ER)-related kinase (PEK/PERK), and the evolutionarily conserved general control nonderepressible-2 (GCN2) kinase. Although each eIF2α kinase shares a conserved kinase domain, they also have unique regulatory domains that allow them to be activated by distinct stimuli[15].

While phosphorylation of eIF2α leads to a general inhibition of translation, it paradoxically results in translational upregulation of a subset of mRNAs.[11–13,15] This unconventional translational control mechanism was first discovered when studying the amino acid control response in yeast.[11,16] In yeast cells, amino acid deprivation leads to the activation of GCN2, which in turn stimulates translation of GCN4 mRNA. The specific induction of GCN4 mRNA translation is mediated by four open reading frames (uORFs) located in the 5′UTR of the mRNA. According to a current model, under nonstarvation conditions, ribosomes scan these short ORFs and rebind the ternary complex and reinitiate translation at the inhibitory uORF4, after which they dissociate from the mRNA before reaching the canonical GCN4 AUG start codon. In contrast, under starvation conditions, eIF2α phosphorylation causes a decrease in ternary complex formation. Consequently, a significant fraction of the scanning ribosomes bypass the inhibitory upstream ORF4, and reinitiate translation at the appropriate GCN4 start codon.[11,17]

A similar mechanism was described in mammalian cells where the translation of the mRNA for the transcriptional modulator ATF4 and the beta-site amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1) is enhanced by eIF2α phosphorylation[18,19] (Fig. 2). Therefore, eIF2α phosphorylation regulates both general and gene-specific translation. Importantly, ATF4 and its homologues play important roles as repressors of synaptic plasticity and memory formation in diverse phyla.[20–22]

II. GCN2-Mediated eIF2α Phosphorylation in Long-Lasting Synaptic Plasticity and LTM Consolidation

As mentioned earlier, eIF2α phosphorylation regulates two fundamental processes that are crucial for the formation of long-lasting memories: new protein synthesis and the memory repressing factor ATF4. The findings that

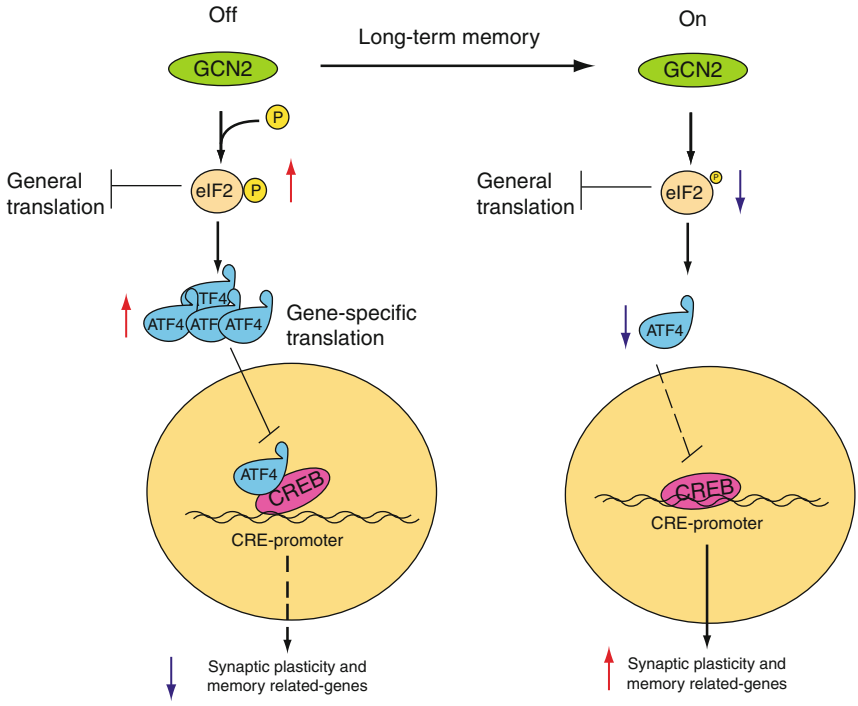

FIG. 2. eIF2α phosphorylation: switching memories ON and OFF. Under basal conditions (OFF), due to partial phosphoryation of eIF2α, general translation is reduced and *ATF4* mRNA translation is augmented. As a consequence, the expression of synaptic plasticity and memory-related genes is blocked. In response to a learning experience (ON), decreased eIF2α phosphorylation reduces *ATF4* mRNA translation and enhances general mRNA translation, thus facilitating the induction of gene expression which leads to long-lasting changes in synaptic strength and long-term memory.

activity-dependent long-lasting changes in synaptic strength either *ex vivo* or *in vivo* decreases the phosphorylation of eIF2α[23–25] raised the interesting possibility that eIF2α acts as molecular switch for the conversion of short-lasting processes into long-lasting one. To test this hypothesis, Costa-Mattioli and colleagues characterized mice with reduced eIF2α phosphorylation: either lacking GCN2, the major eIF2α kinase in the mammalian brain, or heterozygous for a mutation in eIF2α, which converts Ser51 into Ala (eIF2α$^{+/S51A}$).[23,24] eIF2α$^{+/S51A}$ mice exhibited an enhanced LTM in several behavioral tasks including Morris Water Maze, Contextual and Auditory Fear Conditioning and Conditioned Taste Aversion. In contrast, GCN2 knockout mice showed an enhanced spatial memory but only under more demanding training protocols. In contrast, injection with a pharmacological inhibitor of eIF2α phosphatases (Sal003) blocked the formation of LTM storage.[24] These data conclusively demonstrate that eIF2α phosphorylation bidirectionally controls behavioral learning (Fig. 2).

In agreement with the behavioral data, in both genetic models (GCN2 knockout and eIF2α$^{+/S51A}$ mice), ATF4 levels were downregulated and the threshold for eliciting L-LTP was lower, that is, a protocol that usually elicits a short-lasting E-LTP, which is independent of gene expression (translation and transcription), elicited a sustained gene expression-dependent L-LTP.[23] Accordingly, a similar phenotype was observed in mice expressing a dominant negative ATF4 mutant in the forebrain.[22] Indeed, the control of ATF4 levels as a means to modulate memory is an evolutionarily conserved process since the *Aplysia* homologue of ATF4, ApCREB represses long-term facilitation (LTF)[21] and injection of anti-ApCREB2 antibodies into *Aplysia* sensory neurons coupled with a single pulse of serotonin (5-HT, 5-hydroxytryptamine), which normally induces only short-term facilitation (lasting for minutes), is sufficient to evoke a long-lasting gene expression-dependent facilitation. In addition, increased eIF2α phosphorylation in wild-type (WT) hippocampal slices prevents the induction of L-LTP. The impaired L-LTP in slices treated with Sal003 required ATF4 since in ATF4 knockout slices Sal003 failed to suppress L-LTP. These data indicate that the phosphorylation of eIF2α dictates whether a LTM will be generated from a given experience[26] (Fig. 2).

While L-LTP was normal in slices from eIF2α$^{+/S51A}$ mice, a strong stimulation that normally induces L-LTP in WT slices, elicited only a short-lasting LTP in hippocampal slices from GCN2 knockout mice. One possible explanation for the impairment in L-LTP and LTM in GCN2 knockout mice is excessive CREB activity. Indeed, the phosphorylation state of CREB (pCREB) was significantly increased in *GCN2* knockout mice as determined by Western blotting and immuno-staining (Costa-Mattioli, unpublished data). In agreement with this hypothesis, the expression of a constitutively active CREB in the forebrain (a) facilitates the establishment of long-lasting LTP, (b) reduces the amplitude of the L-LTP induced by four tetanic trains,[27] and (c) blocks LTM storage.[28] Thus, neurons appear to have not only a threshold for the activation of gene expression, but also another threshold where too much gene expression blocks synaptic plasticity. This argument becomes even more compelling since this phenotype is shared with the translation inhibitor 4E-BP2 knockout where L-LTP was also impaired with a four tetanic train protocol.

III. eIF2α Phosphorylation and Alzheimer's Disease

Alzheimer's disease (AD) is the most common cause of dementia. According to a recent report, there are 5.3 million Americans living with the disease and by 2050 there will be nearly a million new cases per year. Phosphorylation of eIF2α is associated with neuronal degeneration in AD. In human postmortem hippocampus tissue and the hippocampus from AD mouse models,[29,30] eIF2α phosphorylation is enhanced. In addition, a recent paper supports the idea that

eIF2α phosphorylation not only regulates cognition but may also be important for the AD pathology. O'Connor and colleagues found that increased eIF2α phosphorylation in AD leads to enhance production of BACE1.[31] Interestingly, as expected for mRNA whose translation is stimulated in response to eIF2α phosphorylation, the 5′UTR of BACE1 mRNA contains uORFs, which are evolutionarily conserved. Although, it is currently unclear whether the control of translation of BACE1 mRNA is similar to that of ATF4 or GCN4 mRNAs, in this model increased eIF2α phosphorylation in AD is expected to enhance BACE1 levels and thus amyloid beta production.

IV. Regulation by mTOR

Most of the evidence implicating the mTOR signaling pathway in long-lasting synaptic plasticity and LTM is based on the evidence that rapamycin blocks long-lasting synaptic plasticity in invertebrates[32] and mammalian brain slices.[33,34] Consistent with these data, it also blocks long-term spatial memory formation in mammals.[35,36] Second, in hippocampal slices, several forms of synaptic plasticity activate mTOR and its downstream targets.[33,34,37–40] Third, the PI3K signaling pathway is also critical for LTP in different areas of the brain[41–43] and memory consolidation in the hippocampus and amygdala.[39,41] Finally, deletion of mTOR upstream and downstream targets alters long-term synaptic plasticity and memory. However, some results are conflicting: for instance, $TSC1^{+/-}$-heterozygous mutants and FKBP12 knockout mice result in enhanced mTORC1 activity. However, $TSC1^{+/-}$-mutant mice exhibit impaired contextual and spatial LTM while FKBP12 KO mice exhibit an enhanced contextual but normal spatial memory.

V. Regulation of Protein Synthesis-Dependent Synaptic Plasticity and Memory Consolidation by 4E-BP2 and S6K1 and S6K2

The function of 4E-BP2 in synaptic plasticity has been studied at Schaffer collateral-CA1 pyramidal neuron synapses in the adult hippocampus of 4E-BP2 knockout mice. In the hippocampus of these animals the lack of the translational repressor 4E-BP2 increases the level of the eIF4F complex.[38] As a consequence, 4E-BP2 knockout mice exhibit an enhanced mGluR (metabotropic glutamate receptor)-LTD.[37] Interestingly, rapamycin did not block the enhanced mGluR-LTD in slices from the 4E-BP2 knockout mice, suggesting that 4E-BP2 is the only mTOR downstream target involved in

mTOR-dependent mGluR-LTD. However, mice lacking S6K1 and S6K2, the other major mTOR downstream targets, exhibit enhanced mGluR-LTD[44]. The discrepancy between these results remains to be resolved.

Given that S6K1/2 knock-out mice exhibit normal L-LTP, 4E-BP2 appears to be the major mTOR downstream target regulating late-LTP. The lack of 4E-BP2 facilitates LTP in as much as a protein synthesis-independent E-LTP induces a protein synthesis-dependent L-LTP in slices from the 4E-BP2 knockout mice.[38] However, as was the case for GCN2 knockout mice, an L-LTP stimulation protocol (four trains of HFS) leads to impaired L-LTP in the slices from the 4E-BP2 knockout mice. In correlation to the data, 4E-BP2 knockout mice are impaired in spatial learning in the Morris water maze, and in both auditory and contextual fear conditioning.[38] It would be interesting to determine whether this impairment can be rescued with a weak training paradigm, as was the case for the GCN2 knockout mice.[23] These date indicate that proper translational control of synaptic plasticity and memory genes is critical for mnemonic processes.

VI. Altered mTOR Signaling and Autism Spectrum Disorders

Autism represents a heterogeneous group of disorders that are defined as "autism spectrum disorders" ASDs). ASD individuals exhibit common features such as impaired social interactions, language and communication, and abnormal repetitive behavior. In addition, about 70% of autistic individuals suffer from mental retardation.[45] Although impaired cognition is common in autism, about 10% of ASD individuals exhibit outstanding abilities such as arts, music, calculations, and mathematics. Autism is a heritable genetically heterogeneous disorder. Several single gene mutations are linked to autism. In particular, mutations in upstream regulators of the mTOR signaling pathway are associated with ASD. For instance, the autosomal dominant disorder tuberous sclerosis, which is caused by mutations in the mTOR upstream inhibitors TSC1 or TSC2, results in autism.[46] In addition, PTEN hamartoma syndrome, which is caused by the loss of function in PTEN, an upstream negative regulator of mTOR, has been linked to ASD pathogenesis.[47,48] Neurofibromatosis type I, which is generated by mutations in neurofibromin (NF1), a Ras GAP, results in upregulation of Ras/Erk which in turns leads to the inactivation of TSC2 and subsequent increase in mTOR activity.[49,50] Furthermore, in mouse models, neuron-specific deletion of PTEN or heterozygous TSC2 mutants leads to enhanced mTOR activity and behaviors consistent with autism.[47,51]

Deletion of PTEN or TSC2 in the mouse brain results in macrocephaly,[47,51,52] which is reminiscent of the high prevalence of macrocephaly observed in children with ASDs.[53] Concomitantly, mice lacking the immunophilin FKBP12, which binds to mTOR as a complex with rapamycin, exhibit

increased mTORC1 activity, altered synaptic plasticity, and memory and autistic/obsessive-compulsive-like perseveration phenotypes.[54] Importantly, two recent reports show that chronic treatment with rapamycin rescues the impaired hippocampal memory in TSC and PTEN mutant mice.[47,55] Taken together, these data suggest that upregulation of mTOR leads to autism. It is important to emphasize that it is not just the "molecular" change in mTOR activity *per se* that might cause the ASD-like phenotype but its impact on long-lasting synaptic changes in neural circuits and ultimately long-lasting behavior. Whether mTOR regulates autism through translational control or other mTOR-driven process such as ribosome biogenesis, cell-cycle regulation, or autophagy remains to be determined.

VII. FMRP and Long-Lasting Hippocampal Synaptic Plasticity

Metabotropic glutamate receptor-mediated long-term depression (mGluR-LTD) is a protein synthesis-dependent form of synaptic plasticity that takes place in the synapto-dendritic compartment. It can be induced by *(RS)*-3,5-dihydroxyphenylglycine (DHPG), a selective group I mGluR agonist.[33,56] FMRP, the product of the Fragile X gene, negatively regulates translation during mGluR-LTD.[33,56,57] Moreover, FMRP is translated in response to stimulation of group I mGluRs in synaptosomes (an *in vitro* preparation of synapses), cultured cortical and hippocampal neurons, hippocampal slices, and in the brain *in vivo*.[10] Based on these and other results, Bear *et al*.[58] put forth the "mGluR theory of Fragile X mental retardation," which suggested that disproportionate mGluR-dependent protein synthesis induces the multiple phenotypes that are characteristic of the Fragile X syndrome (FXS). In hippocampal slices, DHPG-induced mGluR-LTD results in the synthesis of FMRP, which depends on the mGluR subtype mGluR5. Somewhat surprisingly, the rapid increase in FMRP is followed by the ubiquitination and destruction of FMRP; conversely, inhibition of the ubiquitin–proteasome pathway abrogates mGluR-LTD, as does the overexpression of FMRP.[57] In wild-type mice, mGluR-LTD is correlated with rapid increases in proteins whose mRNAs are bound by FMRP; such increases are abrogated in *Fmr1* knockout mice. In *Fmr1* knockout mice, but not in WT mice, both protein synthesis[57] and proteasome inhibitors have no effect on mGluR-LTD. These findings suggest that there is an overabundance of translation of normally FMRP-bound mRNAs in *Fmr1* knockout mice and that these mRNAs are translated during mGluR-LTD in WT mice. Such results indicate that rather than an additional level of excessive translation, mGluR-dependent translational control is absent in *Fmr1* knockout mice.

One key question of course is how could excess mGluR-dependent translation takes place in *Fmr1* knockout mice? Perhaps excessive activation of mGluRs occurs in the *Fmr1* knockout mice, which in turn triggers exaggerated activation of translational control pathways. In an important study, Dolen *et al*.[59] demonstrated that *Fmr1* knockout mice heterozygous at the mGluR5 locus do not exhibit several FXS phenotypes. In addition, treatment of *Fmr1* knockout mice with the mGluR5 antagonist MPEP also "rescues" several FXS phenotypes[60]; a similar approach to rescue FXS "phenotypes" in *Drosophila* with mGluR antagonists has been reasonably successful[61]. Moreover, deletion of the FMRP gene in *Drosophila* results in memory deficits that can be restored by protein synthesis inhibitors, consistent with the idea that an overabundance of protein synthesis may be responsible for reduced cognition in FXS.[62] Although an extensive characterization of translational control pathways has yet to occur, phosphorylation of PDK-1, mTOR, and S6K1 by DHPG does not occur in *Fmr1* knockout mice.[63] Similar results have been observed for extracellular signal-regulated kinase,[57] which also is required for translational control in long-lasting hippocampal synaptic plasticity and memory[37,64,65]. Therefore, excessive basal translation and a lack of mGluR-dependent translational control are features that probably contribute to plasticity and behavioral phenotypes displayed by *Fmr1* knockout mice.

FMRP binds many mRNAs such as Arc/Arg3.1, αCaMKII, PSD-95, SAPAP3, and MAP1B and increased expression of transcripts occurs in the brains or cultured neurons from *Fmr1* knockout mice.[57,66–69] Consistent with studies under conditions where FMRP is reduced,[70] mGluR-LTD in hippocampal slices is associated with protein synthesis-dependent increases in the levels of FMRP, MAP1B, αCaMKII, and Arc/Arg3.1[57,71]. Although the consequences of increased αCaMKII in mGluR-LTD are not clear, increased MAP1B and Arc/Arg3.1 proteins are required for mGluR-dependent endocytosis of AMPA receptors[70–72]. In addition, enhanced translation of Arc/Arg3.1 also is required for the expression of mGluR-LTD.[71] Taken together, these studies indicate that translation of FMRP-bound mRNAs contributes to mGluR-LTD and suggest that excessive basal translation of these mRNAs might contribute to the plasticity and behavioral phenotypes observed in FXS.

VIII. Translational Control by FMRP

The molecular mechanism by which FMRP modulates translation has been intensively studied but remains controversial. Although there is a general consensus that FMRP inhibits translation, some evidence is consistent with it being a translational activator. For example, several investigators find that a substantial amount of FMRP sediments with polysomes,[73–75] which would be

expected of an activator of translation, and indeed Ceman *et al.*[76] suggest that FMRP does just that. In addition, metabolic labeling of protein in the hippocampus of *Fmr1* knockout mice exceeds that of wild-type mice,[59] again indicating that FMRP inhibits translation. However, it is also possible that FMRP activates the translation of some mRNAs at early times of, say, development or following synaptic stimulation, which causes subsequent mRNA-specific translational repression. That FMRP might repress but also activate mRNAs is suggested by the results of Brown *et al.*[77], who found that of several mRNAs that coimmunoprecipitated with FMRP, some sedimented to heavy polysomes while others shifted to light polysomes in cells lacking FMRP. Thus, FMRP could either be bifunctional—that is, repressing some mRNAs while activating others, or affect all mRNAs the same way (say, repression), which is followed by a cascade of translational control that is both repressing and activating.

A recent intriguing model has been proposed for how FMRP regulates translation that has broad implications for translational control in general. Napoli *et al.*[75] reported that a large portion of FMRP sediments in fractions lighter than polysomes; such fractions also contain CYFIP1 (cytoplasmic FMRP interacting protein), a factor that binds FMRP, as well as eIF4E. Surprisingly, CYFIP1 and FMRP are both retained on m^7GTP (cap)-Sepharose columns, indicating that they directly or indirectly bind the cap. Because both proteins are competed off the column by excess 4E-BP, Napoli *et al.* surmised that FMRP and CYFIP bind the cap through an interaction with eIF4E; they further showed that it is CYFIP1 that directly binds eIF4E. CYFIP1 contains a region with some similarity to eIF4E binding proteins, but surprisingly, does not conform to the $YXXXXL_{\Phi}$ (where Φ is any hydrophobic amino acid, often a leucine) sequence that is common among such proteins[78]. Instead, CYFIP1 has a "noncanonical" sequence that is predicted to form two helices that are nearly identical in structure to those formed by the consensus eIF4E binding peptide[75,79]. The CYFIP1 α helices are stabilized by predicted internal salt bridges and indeed the residues that are thought to form these bridges are necessary for CYFIP1's interaction with eIF4E.[75] eIF4E–CYFIP1–FMRP complexes can be detected in synaptoneurosome preparations, and upon synaptic stimulation, the CYFIP1–eIF4E interaction is destroyed and FMRP-bound mRNAs undergo enhanced translation.

These results[75] indicate that at least one mode of FMRP-inhibited translation is analogous to that of CPEB. That is, an RNA binding protein (FMRP or CPEB) is bound to an eIF4E-associated factor (CYFIP1 or Maskin) to preclude the recruitment of eIF4G, and indirectly the 40S ribosomal subunit, to the 5′ end of the mRNA.[80] One may also infer that molecules with "Maskin-like" activities, that is, mRNA-specific 4E-BPs, may be more widespread than previously thought. For example, *Drosophila* cup[81], mammalian 4E-T[82], and mammalian neuroguidin[83] all contain the $YXXXXL_F$ motif noted earlier and

thus resemble Maskin (although Maskin has a threonine in place of the tyrosine); CYFIP1, however, may be the first among other soon-to-be-discovered molecules with "noncanonical" eIF4E binding regions that could regulate translation of many sets of mRNAs by associating with different RNA binding proteins.

In addition to the mechanism by which FMRP affects translation, the sequence(s) to which it binds is also complex owing to the fact that the protein contains 2 KH (RNP K homology) domains and a RGG box. FMRP has been reported to bind an unusual intramolecular duplex structure known as a G-quartet through the RGG box,[84] a small noncoding ds RNA (BC1) via a previously undescribed RNA binding motif,[68,85] and a loop–loop pseudoknot "kissing complex" via KH domain 2.[73] However, the only portion of FMRP linked to the FXS is KH domain 2; one individual with an I304N mutation within this region displays several characteristics of the syndrome. Interestingly, the kissing complex, when added in trans, induces a large shift in the sedimentation profile of FMRP such that it is almost exclusively in the mRNP fraction. This result draws a clear connection between the FXS, FMRP KH domain 2, and the kissing complex. Of course, "the absence of evidence is not evidence of absence," and the other domains of FMRP and the RNAs to which they bind could also contribute to the FXS.

IX. The Exon Junction Complex and the Regulation of Synaptic Strength

The exon junction complex (EJC) consists of four core proteins that are probably deposited on most, if not all, exon–exon junctions following intron removal from nuclear pre-mRNA. The four proteins, eIF4AIII, Y14, Mago, and MLN51 travel with the mRNA as it is exported to the cytoplasm where they help dictate the fate of the transcript. Although it is generally thought that the first, or pioneer round of translation causes the dissociation of the EJC from mRNA, prior to this event, this complex can regulate mRNA translation, localization, and destruction in conjunction with other ancillary proteins.[86–89] These processes are often interconnected; for example, the EJC can stimulate translation before it dissociates from the mRNA during the first "pioneer round" of translation that is important for RNA quality control. Together with Upf1 and other factors, the EJC can influence nonsense-mediated mRNA decay (NMD), a surveillance mechanism to ensure that mRNAs with aberrant stop codons are destroyed and do not make improper proteins that could be deleterious to cells.

As noted earlier, translation at synapses is regulated by several factors including mTOR and its effectors and FMRP. Certainly additional translational control mechanisms/factors operate at synapses, and Giorgi *et al*.[87] have proposed an intriguing new one. They noted that eIF4AIII, the EJC component, displays a dendritic as well as cell body localization in cultured hippocampal and cortical neurons (in tissue culture cells such as Hela, the preponderance of eIF4AIII is nuclear), and interacts with some dendritic mRNAs such as that encoding Arc/arg3.1. Arc/arg3.1 is an immediate early gene whose transcription is induced by a variety of agents and behaviors in the hippocampus,[71] all of which probably lead to the activation of *N*-methyl-D-aspartate receptors.[90] The arc/arg3.1 3′UTR is formed from three exons, and thus would be expected to have two EJC complexes; hence, the observed coimmunoprecipitation of this mRNA with eIF4AIII. From this observation, Giorgi *et al*. surmised that arc/arg3.1 mRNA could be transported to the synapto-dendritic compartment in a translationally dormant form accompanied by the EJC, and that upon synaptic stimulation, a transient burst of arc/arg3.1 protein synthesis would occur, followed soon thereafter by destruction of the mRNA. Because arc/arg3.1 mRNA EJCs would be located in the 3′UTR, one or a few pioneer rounds of translation presumably would not induce dissociation of the EJC from the mRNA yet the mRNA may still be subject to NMD-like destruction. In this scenario, protein synthesis at synapses would be highly regulated, since after very little translation, the RNA would be destroyed. The evidence that this is the case rests primarily on the fact that a knockdown of eIF4AIIIA in cultured neurons leads to increased levels of dendritic arc/arg3.1 protein and RNA levels. The eIF4AIII knockdown also induces increased excitatory synaptic strength, most likely via the addition of glutamate receptors at synapses.[87]

While attractive, the model[87] would seem to be inconsistent with other observations of arc/arg3.1 mRNA and protein distribution *in vivo*. For example, certain behaviors in rats lead to substantial arc/arg3.1 protein levels in hippocampal cell bodies, indicating that the mRNA is not repressed in that location[91]. Similar observations are made when the rat hippocampus is subjected to electrical stimulation that induces LTP.[92,93] It thus remains unclear whether, or to what extent, arc/arg3.1 mRNA may be transported in dendrites in an inactive form.

X. CPEB-Regulated Molecular Circuitry

CPEB is a sequence-specific RNA binding protein that stimulates translation by inducing cytoplasmic poly(A) elongation.[80] In neurons, CPEB is found at postsynaptic sites (as well as the cell body) where in response to synaptic activity, it induces polyadenylation and translation of several mRNAs[94–97].

The importance of this protein for translation in the brain was demonstrated in a CPEB knockout mouse where theta burst-induced LTP was reduced in hippocampal Shaffer CA-1 neurons[98]. In addition, CPEB knockout mice have a deficit in extinction, a type of memory where behavioral responses diminish and eventually become extinct when there is no reinforcement of the memory.[99] Although extinction requires the formation of new memories, the underlying mechanisms by which it occurs are probably distinct from those of memory acquisition and consolidation[99a].

The key to understanding how CPEB might influence these complex phenotypes surely lies in the identification of target mRNAs. To this end, Zearfoss *et al.*[100] have identified growth hormone (GH) as one protein whose level is reduced ~ 10-fold in the CPEB KO hippocampus. GH mRNA contains no 3′UTR cytoplasmic polyadenylation elements (CPEs), the binding sites for CPEB, and both GH mRNA and pre-mRNA are reduced in the KO versus wild-type hippocampus. This result suggested that an mRNA encoding a transcription factor that regulates GH gene expression might be under the direct control of CPEB. Indeed, c-jun is just such a factor; it is reduced in the hippocampus of CPEB knockout mice, its 3′UTR contains CPEs, and it coimmunoprecipitates the promoter of the GH gene in wild-type but not CPEB knockout mice. Surprisingly, GH itself induces LTP in hippocampal slices which, like electrical stimulation, is reduced in the CPEB knockout mouse. Moreover, the LTP induced by GH and theta burst stimulation is reduced if slices are incubated with cordycepin, a drug that inhibits polyadenylation. These and other results suggest that GH acts in both autocrine and paracrine fashion to regulate plasticity through CPEB control of c-jun mRNA translation.

CPEB is also found in invertebrates, and in Aplysia sensory neurons where CPEB RNA has been ablated by an antisense oligonucleotide, LTF, a form of plasticity, is not properly maintained.[101] However, the isoform of CPEB in Aplysia neurons differs from the CPEB described earlier in mammals in that it contains a long stretch of glutamine residues. Polyglutamine is sometimes found in proteins that have characteristics of a prion, an infectious agent consisting entirely of protein that is self-reproducing. This observation, plus the fact that CPEB RNA is detected in Aplysia neurons suggested to Si *et al.*[101,102] that this CPEB isoform might assume a prion-like structure following synaptic stimulation, thereby forming a protease-resistant tag at synapses. If so, then perhaps CPEB itself, as opposed to proteins derived from CPEB-stimulated translation, might comprise the tag that is thought to distinguish stimulated from naive synapses. Si *et al.*[102] indeed showed that Aplysia CPEB had some features of a prion *in vitro*, such as resistance to protease and fast sedimentation rate in sucrose gradients. The most compelling evidence, however, comes from experiments in yeast, where Alpysia CPEB was

shown to assume two forms: one that is aggregated (i.e., prion-like) and one that is not.[102] Surprisingly, not only was the aggregated form of CPEB the only one to bind RNA *in vitro*, but they also converted the nonaggregated into an aggregated form. Such epigenetic inheritance is a fundamental hallmark of prion formation. Si *et al.*[102] hypothesized that synaptic stimulation might cause the neuronal Aplysia CPEB isoform to assume a prion-like state, which could stimulate the translation of some RNAs, cause it to alter its substrate specificity, or release some mRNAs from an inhibited state. These authors further suggest that once in a prion form, CPEB would need no further stimulation (e.g., by kinases) to maintain its activity.

If polyglutamine-containing CPEB forms a prion in invertebrate neurons, then what about the polyglutamine-lacking CPEB in vertebrate neurons? Vertebrates contain three additional CPEB-like genes, all of which are expressed in the brain.[103] Two of these other CPEB-like proteins do contain some polyglutamine, although they are not nearly as long as that in the Aplysia CPEB. Moreover, these other CPEB-like proteins do not have a strong affinity for the CPE and do not support cytoplasmic polyadenylation.[104] Thus, the relationship between vertebrate CPEB proteins and prions, if any, remains to be determined. Nonetheless, it is noteworthy that the *Drosophila* CPEB isoform called Orb2 is found in a head structure (the mushroom bodies) that is important for LTM and contains polyglutamine; when the polyglutamine is deleted, LTM, but not STM is impaired.[105] While these data do not indicate whether prion formation, or even mRNA translation was involved, they do point to the importance of the glutamine stretch for CPEB isoform function in memory formation.

XI. Perspective

The foregoing treatise has recounted some of the salient events leading to the conclusion that activity-dependent mRNA translation is one of several molecular events that underlie synaptic plasticity and learning and memory. So where will new avenues of investigation lead? One almost certainly lies in the concept of "local" translation. That is, there is considerable evidence that the synaptic-dendritic compartment can translate mRNAs, but it is not yet completely evident that this local translation is essential for synaptic plasticity, and it is completely unknown whether it is required for learning and memory. A second concept sure to gain momentum is miRNA involvement in plasticity. Indeed, one miRNA, mi138, has already been shown to affect synaptic spine morphogenesis.[106] There will very likely be additional miRNAs that affect all aspects of plasticity and it will be very interesting to learn how they coordinate, or oppose, the activities of some of the translational control proteins noted in this chapter.

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