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Translational Control of Synaptic Plasticity and Learning and Memory

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ONE HALLMARK OF LONG-TERM MEMORY CONSOLIDATION is the requirement for new gene expression. Although memory formation has largely focused on transcriptional control (Kandel 2001), it has been known for more than four decades that it also requires protein synthesis (Flexner et al. 1963). This and other early studies offered little in the way of molecular mechanisms because they relied mostly on the injection of general translation inhibitors into animals. The last 10 years, however, have witnessed major advances in our understanding of translational control of memory and its cellular foundation, synaptic plasticity. In this chapter, we discuss the most salient aspects of translational control of these essential brain activities and present our thoughts on some of the key issues remaining to be elucidated.

TEMPORAL PHASES OF SYNAPTIC PLASTICITY AND MEMORY

How are memories stored at the cellular level? Most neuroscientists hypothesize that memory involves changes in the strength of synaptic connections between neurons (i.e., synaptic transmission). These changes in synaptic efficacy are referred to as *synaptic plasticity* and are manifested as either an increase (potentiation) or decrease (depression) in strength.

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Long-term potentiation (LTP) and long-term depression (LTD) have been intensively studied in the rodent hippocampus, a brain structure that is critical for processing information about space, time, and the relationship between objects. Both LTP and LTD can be induced routinely in vitro with distinct patterns of electrical stimulation delivered to synapses in preparations of hippocampal slices.

More than 20 years ago, hippocampal LTP was shown to require new protein synthesis in vivo (Krug et al. 1984). A number of subsequent studies using transcription and translation inhibitors demonstrated that LTP has distinct temporal phases (Frey et al. 1988; Huang and Kandel 1994; Nguyen et al. 1994): The phase that is induced by a single train of high-frequency stimulation (HFS) lasting for 1–2 hours is referred to as the “early phase” LTP (E-LTP), whereas the phase that is induced by multiple, spaced trains of HFS persisting for 3 or more hours is referred to as the “late phase” LTP (L-LTP). Mechanistically, these two forms of LTP differ with respect to their requirement for new mRNA and protein synthesis. L-LTP is sensitive to both transcription and translation inhibitors, whereas E-LTP is not sensitive to either. Temporal phases of hippocampal LTP also have been observed when LTP is induced with θ -burst stimulation, a type of stimulation protocol that more closely resembles hippocampal neuron activity in vivo (Nguyen and Kandel 1997). Finally, distinct temporal phases have been described for synaptic plasticity in invertebrate systems such as long-term facilitation (LTF) at the sensorimotor synapse in *Aplysia* (Kandel 2001).

The induction of both E-LTP and L-LTP requires activation of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor. Additional protein-synthesis-dependent forms of LTP can be induced in hippocampal slices with a number of receptor agonists, including the neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (Kang and Schuman 1996), activators of the cAMP signaling pathway (Frey et al. 1993), and those that activate dopamine receptors (Huang and Kandel 1995). Moreover, long-lasting protein-synthesis-dependent forms of hippocampal LTD have also been described. Activation of group I metabotropic glutamate receptors (mGluRs) via direct pharmacological activation with the agonist 3,5-dihydroxyphenylglycine (DHPG) or with paired-pulse low-frequency stimulation (PP-LFS) results in protein-synthesis-dependent L-LTD (Huber et al. 2000). Protein-synthesis-dependent LTD also can be triggered by insulin (Huang et al. 2004). Finally, LFS induces NMDA-receptor-dependent LTD (Dudek and Bear 1992; Mulkey and Malenka 1992) that is blocked by protein synthesis inhibitors (Kauderer and Kandel 2000; Sajikumar and Frey 2004). Thus, protein-synthesis-

dependent LTP and LTD can be induced with via activation of multiple receptors in the hippocampus.

Similar to synaptic plasticity, memory also exhibits distinct temporal phases that can be differentiated by transcription and translation inhibitors. A good illustration of this type of differentiation is the gill-withdrawal reflex in *Aplysia*, which is associated with synaptic facilitation. A single sensitizing stimulus results in short-term memory that depends on covalent modifications to existing proteins and is insensitive to transcription and translation inhibitors; conversely, multiple, spaced sensitizing stimulation results in long-term memory that is blocked by those inhibitors (Castellucci et al. 1989). Similar observations have been made in rodents where, in general, new mRNA and protein synthesis is required for long-term memory but not short-term memory (McGaugh 2000; Kandel 2001). Later in this chapter, we discuss specific mechanisms of translational control that mediate long-term memory in rodents.

CONTRIBUTION OF LOCAL PROTEIN SYNTHESIS TO SYNAPTIC PLASTICITY

Neuronal dendrites and dendritic spines contain polyribosomes (Steward and Levy 1982), translation factors (Tang et al. 2002), and mRNA that can be translated into protein at synapses (Crino and Eberwine 1996). Imaging studies with fluorescent reporters have shown that both BDNF and specific manipulations of neuronal activity trigger protein synthesis in dendrites (Aakulu et al. 2001; Ju et al. 2004; Sutton et al. 2004). Local (i.e., synaptic) protein synthesis also has been demonstrated to be necessary for BDNF-induced potentiation and mGluR-dependent LTD in the hippocampus (Kang and Schuman 1996; Huber et al. 2000). Protein synthesis inhibitors block both of these types of synaptic plasticities even when the cell bodies of the hippocampal neurons are severed from their dendrites. Local protein synthesis also is required for LTF in *Aplysia* sensory neurons and plasticity at nerve–muscle synapses in *Xenopus* (Martin et al. 1997; Zhang and Poo 2002). These findings demonstrate that local protein synthesis is required for numerous types of synaptic plasticities.

Emerging evidence indicates that local protein synthesis is required for hippocampal LTP. As mentioned earlier, L-LTP induced with multiple trains of HFS requires both transcription and translation. However, the translation inhibitors affect L-LTP at earlier times than the transcription inhibitors (Kelleher et al. 2004b; Banko et al. 2005), implying that L-LTP itself consists of an early translation-dependent phase that is independent of transcription followed by a transcription- and transla-

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tion-dependent phase. The translation-dependent early phase of L-LTP is presumably due to local protein synthesis. Consistent with this idea, a translation-dependent, transcription-independent LTP has been shown in isolated dendrites in hippocampal slices (Cracco et al. 2005; Vickers et al. 2005). Furthermore, local application of a protein synthesis inhibitor to dendrites blocks L-LTP (Bradshaw et al. 2003). Thus, local protein synthesis is required for the earliest phase of L-LTP.

GENERAL TRANSLATIONAL CONTROL MECHANISMS DURING SYNAPTIC PLASTICITY

As described in Chapter 4, the integrity of the eukaryotic initiation factor 4F (eIF4F) cap-binding complex is modulated by eIF4E-binding proteins (4E-BPs). Hypophosphorylated 4E-BPs bind to eIF4E and inhibit translation whereas their hyperphosphorylated forms do not, thereby permitting eIF4F complex formation and translation initiation. Biochemical studies have shown that both LTP and mGluR-LTD are associated with enhanced 4E-BP phosphorylation (Kelleher et al. 2004b; Banko et al. 2005, 2006; Schmitt et al. 2005). 4E-BP phosphorylation is regulated by extracellular-signal-regulated kinase (ERK), phosphoinositide 3-kinase (PI3K), and mammalian target of rapamycin (mTOR) pathways, and it is known that mTOR phosphorylates 4E-BP (Gingras et al. 1998; Chapter 14). The ERK (Sweatt 2004; Thomas and Huganir 2004) and PI3K (Kelly and Lynch 2000; Beaumont et al. 2001; Sanna et al. 2002; Opazo et al. 2003) signaling cascades are required for a plethora of types of synaptic plasticities, and recent studies have demonstrated that mTOR activity is required for several protein-synthesis-dependent forms of synaptic plasticity. For example, the mTOR inhibitor rapamycin blocks LTF in *Aplysia* sensory neurons (Casadio et al. 1999) and at the crayfish neuromuscular junction (Beaumont et al. 2001). In the rodent hippocampus, rapamycin blocks mGluR-LTD (Hou and Klann 2004), insulin-induced LTD (Huang et al. 2004), BDNF-induced potentiation (Tang et al. 2002), and L-LTP (Tang et al. 2002; Cracco et al. 2005; Tsokas et al. 2005; Vickers et al. 2005). Thus, mTOR-dependent regulation of 4E-BP phosphorylation is a major component of protein-synthesis-dependent synaptic plasticity (Fig. 1).

In the mouse hippocampus, 4E-BP2 is the predominant 4E-BP isoform (Banko et al. 2005). LTP and mGluR-LTD have been examined in 4E-BP2 knockout mice; in hippocampal slices from these animals, protein synthesis-independent E-LTP is converted to protein-synthesis-dependent L-LTP and is correlated with increased eIF4F complex forma-

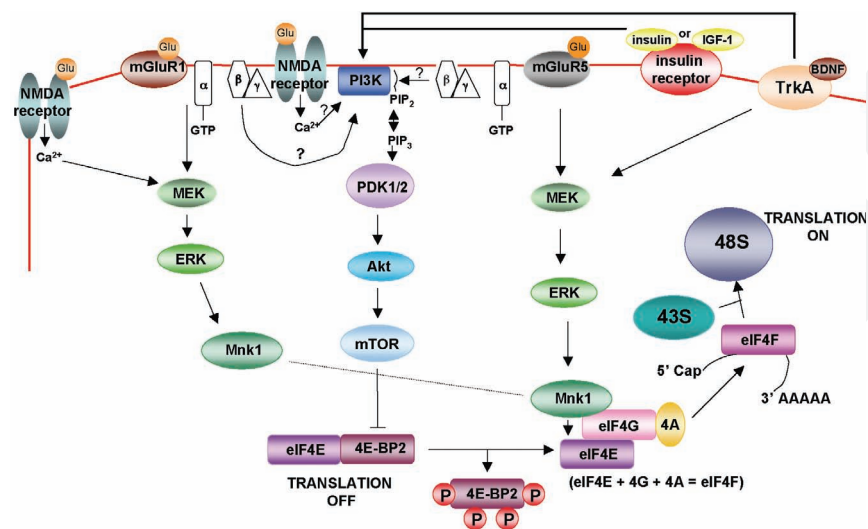


Figure 1. Signaling pathways that control translation initiation during various forms of LTP and LTD. Pharmacological, biochemical, electrophysiological, and genetic evidence indicates that activation of NMDA receptors and TrkA receptors results in LTP, whereas activation of group I mGluRs and insulin receptors results in LTD. Activation of MEK/ERK and PI3K/Akt is necessary for both types of synaptic plasticities. PI3K/Akt-dependent activation of mTOR results in the phosphorylation of 4E-BP2, which causes the dissociation of 4E-BP2 from eIF4E. This permits eIF4E to bind to eIF4G, resulting in the formation of the active eIF4F complex, which is a necessary component of the 48S initiation complex. Mnk1, which also binds eIF4G, is phosphorylated and activated by ERK. Mnk1 phosphorylates eIF4E, an event that is associated with increased translation rates. The eIF4F complex and the poly(A) tail act synergistically to stimulate mRNA translation. There is evidence that similar cascades are activated during LTF via serotonin-dependent signaling in *Aplysia* sensory neurons.

tion (Banko et al. 2005). In addition, enhanced mGluR-LTD is observed in the 4E-BP2 knockout mice (Banko et al. 2006). Taken together, these findings might suggest that the lack of 4E-BP2, which results in increased eIF4F complex formation, induces a robust but general synaptic plasticity in the hippocampus. On the other hand, L-LTP is abrogated in the 4E-BP2 knockout mice even though additional eIF4F complex formation is detected. Consequently, enhanced eIF4F complex formation and possibly excessive translation initiation may be detrimental to L-LTP (Banko et al. 2005). Interestingly, LTP-dependent increases in eIF4F complex formation also were observed in 4E-BP2 knockout mice, which strongly suggests that additional eIF4E-binding proteins regulate translation initia-

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tion during hippocampal LTP. In contrast, additional eIF4F complex formation was not observed in association with mGluR-LTD in 4E-BP2 knockout mice (Banko et al. 2006), suggesting that 4E-BP2 is the only eIF4E-binding protein regulated during mGluR-LTD.

eIF4E is a target for direct phosphorylation (Chapter 14), which could be important for synaptic plasticity. The neurotransmitter serotonin, which is required for LTF in *Aplysia* sensory neurons, induces a p38 MAP kinase-dependent increase in eIF4E phosphorylation (Dyer and Sossin 2000). ERK-dependent increases in eIF4E phosphorylation are induced in cultured neurons treated with BDNF (Kelleher et al. 2004b) and in hippocampal slices treated with NMDA (Banko et al. 2004). Moreover, increased eIF4E phosphorylation has been observed during several forms of synaptic plasticities; electrical stimulation that induces either E-LTP (Banko et al. 2005; Schmitt et al. 2005) or L-LTP (Kelleher et al. 2004b; Banko et al. 2005) triggers ERK-dependent increases in eIF4E phosphorylation. Finally, it recently was shown that mGluR-LTD is associated with an ERK-dependent increase in eIF4E phosphorylation (Banko et al. 2006). Although none of these studies directly demonstrates a requirement of eIF4E phosphorylation for protein-synthesis-dependent synaptic plasticity, they suggest that multiple forms of synaptic plasticities are associated with regulation of a critical translation factor required for cap-dependent translation.

Of course, translation initiation is regulated via other translation factors and protein kinases, such as eIF2 α and its kinases, which are likely to be important for protein-synthesis-dependent synaptic plasticity. Regulation of eIF2 α by the protein kinase GCN2 during hippocampal LTP and memory is discussed later in this chapter.

The data described above indicate that the signaling mechanisms used to regulate cap-dependent initiation are very similar for most forms of synaptic plasticity. How, then, can such signaling lead to translational control mechanisms that result in LTP versus LTD? One possibility is that there is mRNA-specific translational regulation, a topic discussed later in this chapter.

TRANSLATIONAL CONTROL OF LEARNING AND MEMORY

As noted earlier, long-term memory, but not short-term memory, requires mRNA and protein synthesis (McGaugh 2000; Kandel 2001). Translational control mechanisms that are involved in the formation of long-term memory are largely unknown, but several recent studies indicate that mechanisms that are required for hippocampal synaptic plasticity also are required for hippocampus-dependent long-term memory.

Two standard tests of hippocampal-dependent memory in rodents are platform location in the Morris water maze (spatial memory) and “freezing” in response to a mild foot shock (contextual fear memory). The Morris water maze measures the ability of an animal to learn and remember the relationship between multiple visual cues and the location of a hidden platform in a pool of opaque water (Morris 1984). A typical training protocol entails two blocks of four training trials per day with an interblock interval of 1 hour; this procedure would be repeated each day for 7 days. The time an animal takes to reach the platform is measured; it is referred to as escape latency and is one index of learning ability. Not surprisingly, the escape latency of an animal in the Morris water maze test usually decreases over the course of the training period. To assess spatial memory, the platform is removed from the pool and the animals are allowed to search for 60 seconds (usually 1 hour after the completion of the last training trial). In these probe trials, the time spent searching in each quadrant measures the spatial search strategy of the animal (Schenk and Morris 1985). In addition, platform crossings, the number of times an animal crosses the exact place where the platform had been located, are also noted.

The hippocampus is also involved in contextual fear memory. In this case, a conditioned fear paradigm is employed in which animals learn to fear a new environment because of its association with an aversive unconditioned stimulus such as a foot shock. When animals are exposed to the same context at a later time, those that are “conditioned” demonstrate several stereotypical fear responses such as freezing (Fanselow 1984; Phillips and LeDoux 1992).

Spatial memory has been examined in both the 4E-BP2- and GCN2-deficient mice. The 4E-BP2 knockout mice exhibit impaired spatial learning as measured by higher escape latencies during training, and impaired spatial memory as measured by both time spent in the target quadrant and the number of platform crossings (Banko et al. 2005). GCN2 knockout mice exhibit similar impairments in spatial learning and memory (Costa-Mattioli et al. 2005). Interestingly, when the GCN2 knockout mice were given a weak training regimen consisting of only one training trial per day, they exhibited enhanced learning and memory (Costa-Mattioli et al. 2005). These findings correlate nicely with the LTP studies in these animals where E-LTP was converted to L-LTP, but L-LTP was inhibited. Taken together, these findings suggest that proper translational control is required for hippocampus-dependent spatial learning and memory.

The 4E-BP2 knockout mice exhibit normal short-term contextual fear memory but impaired long-term contextual fear memory (Banko

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et al. 2005). Similarly, the GCN2 knockout mice also exhibit impaired long-term contextual fear memory (Costa-Mattioli et al. 2005). Thus, in addition to its role in spatial learning and memory, proper translational control also is required for contextual fear memory.

What are the upstream biochemical signaling mechanisms that regulate translation during memory formation? One possibility is the ERK signaling cascade, which is required for most forms of synaptic plasticities and long-term memory (Sweatt 2004; Thomas and Huganir 2004). Consistent with a role for ERK hippocampus-dependent memory, mice expressing a dominant-negative MEK (the upstream kinase that phosphorylates and activates ERK) have impaired long-term contextual fear memory (Kelleher et al. 2004b). Moreover, contextual fear conditioning results in increased phosphorylation of eIF4E that is absent in the dominant-negative MEK mice (Kelleher et al. 2004b). Many more studies will be required to delineate the signaling cascades that regulate translation factor function during both contextual fear memory and spatial memory in the hippocampus.

GENE-SPECIFIC TRANSLATIONAL CONTROL MECHANISMS IN SYNAPTIC PLASTICITY AND MEMORY

eIF2 α Kinases and uORFs

As mentioned in Chapter 12, there are four eIF2 α kinases, all of which have been found in the brain. The eIF2 α kinases, which inhibit general protein synthesis during cellular stress by phosphorylating eIF2 α , can also stimulate translation of mRNAs containing upstream open reading frames (uORFs), such as that encoding the transcription factor ATF4 (Harding et al. 2000; Vattem and Wek 2004). Interestingly, ATF4 is an inhibitor of synaptic plasticity and memory via its antagonism of the cyclic-AMP-response element (Yin et al. 1994; Bartch et al. 1995; Abel et al. 1998; Chen et al. 2003). Recent studies indicate that the eIF2 α kinase GCN2 regulates ATF4 during synaptic plasticity in the hippocampus. GCN2-deficient mice exhibit LTP phenotypes similar to those of 4E-BP2-deficient mice; i.e., E-LTP is converted to protein-synthesis-dependent L-LTP and L-LTP is inhibited (Costa-Mattioli et al. 2005). The basal levels of eIF2 α phosphorylation and the amount of ATF4 were decreased in the GCN2-deficient mice, and these decreases corresponded to enhanced CREB (cAMP-responsive element binding) function as measured by the expression of immediate-early genes regulated by CREB (Costa-Mattioli et al. 2005). These findings suggest that under resting conditions, GCN2

normally represses CREB-dependent transcription and that this suppression is removed when GCN2 is absent. In contrast to LTP, mGluR-LTD, which is enhanced in 4E-BP2-deficient mice (Banko et al. 2006), was unaffected in GCN2-deficient mice (Costa-Mattioli et al. 2005). Thus, the regulation of CREB-dependent transcription via GCN2-dependent ATF4 translation is specific to LTP.

CPEB and Cytoplasmic Polyadenylation

Cytoplasmic polyadenylation is one mechanism that governs specific gene expression in response to synaptic stimulation. Here, dormant mRNAs have relatively short poly(A) tails, usually about 20–40 nucleotides in length. In response to synaptic stimulation, the poly(A) tails on a number of mRNAs lengthen and translation ensues (Wu et al. 1998; Wells et al. 2001; Huang et al. 2002; Shin et al. 2004; Du and Richter 2005). Although most aspects of the molecular mechanism of polyadenylation-induced translation have been elaborated using *Xenopus* oocytes as they progress through the final stages of meiosis (maturation) in preparation for fertilization, most features of this process appear to be conserved in neurons. Two *cis* elements in the 3'-untranslated regions (UTRs) of responding mRNAs are necessary for polyadenylation: the cytoplasmic polyadenylation element (CPE, general structure of UUUUUAU) and the hexanucleotide AAUAAA, which is also important for nuclear pre-mRNA cleavage and polyadenylation (Fox et al. 1989; McGrew et al. 1989). CPEB is an RNA-recognition motif (RRM) and zinc-finger-containing protein that is the central player in cytoplasmic polyadenylation: It not only has a strong affinity for the CPE (Hake and Richter 1994; Hake et al. 1998), but also associates with a number of other key regulatory factors. These factors include symplekin, a scaffold-like protein upon which the polyadenylation machinery is assembled; cleavage and polyadenylation specificity factor (CPSF), a group of four proteins that binds the AAUAAA; and Gld2 (Barnard et al. 2004), an unusual poly(A) polymerase first discovered in yeast and *Caenorhabditis elegans* (Read et al. 2002; Saitoh et al. 2002; Wang et al. 2002). Polyadenylation is triggered by the phosphorylation of S174 or T171 (species-dependent) by the kinase Aurora A (Mendez et al. 2000b; Huang et al. 2002), although some evidence suggests that α CaMKII (calmodulin-dependent protein kinase II) can also phosphorylate this residue (Atkins et al. 2004). In mammalian hippocampal neurons, NMDA receptor activation triggers CPEB phosphorylation (Fig. 2) (Huang et al. 2002). Although phosphorylated CPEB has an enhanced affinity for CPSF (Mendez et al. 2000a), it is unclear

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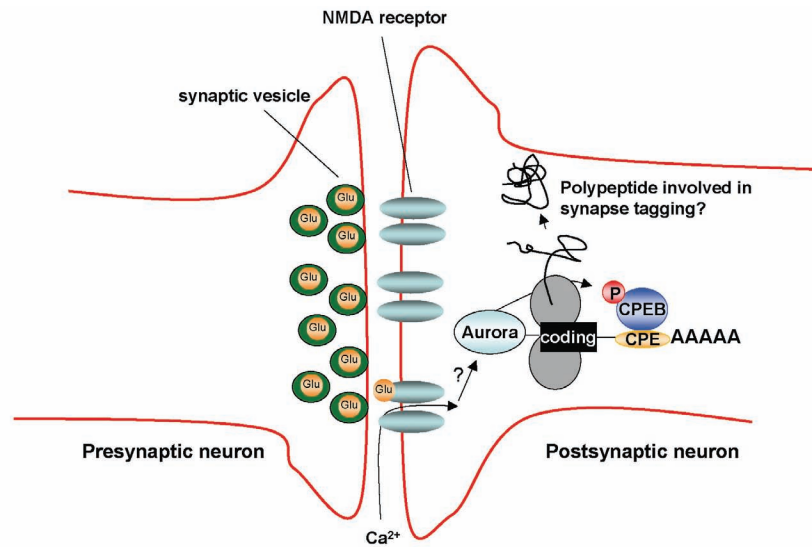


Figure 2. CPEB regulation of synaptic translation. Following synaptic stimulation, calcium enters the postsynaptic neuron via NMDA receptors. This process leads to the activation of the kinase Aurora A, which in turn phosphorylates CPEB and activates the cytoplasmic polyadenylation machinery. Polyadenylation induces the translation of specific (CPE-containing) mRNAs, whose products may be involved in synaptic tagging.

how this event stimulates polyadenylation. In any event, polyadenylation induces translation through other molecules, particularly Maskin, a CPEB-associated factor that also binds the cap-binding factor eIF4E (Stebbins-Boaz et al. 1999). The binding of Maskin to eIF4E precludes the assembly of the eIF4F initiation complex, thus preventing translation (Richter and Sonenberg 2005). The elongated poly(A) tail is bound by poly(A)-binding protein (PABP), which in turn binds eIF4G (Wakiyama et al. 2000); this interaction appears to enhance an eIF4G–eIF4E association at the expense of the Maskin–eIF4E association (Cao and Richter 2002). Consequently, eIF4F assembly on the cap takes place and translation proceeds. Another factor involved in CPEB-mediated translation is p54 (also referred to as DDX6), an RNA helicase (Minshall et al. 2001). Finally, at least a portion of CPEB is anchored to the plasma membrane through members of the amyloid precursor protein family (Cao et al. 2005) and possibly a guanine nucleotide exchange factor as well (Martinez et al. 2005). These proteins stimulate CPEB phosphorylation and resulting polyadenylation.

Aside from these molecular details, does this process influence local translation and synaptic plasticity? A number of experiments indicate that

this is the case. First, CPEB and several of its associated factors (see above) are localized postsynaptically, where local translation most likely occurs (Huang et al. 2002). Second, a CPEB knockout mouse shows a deficit in hippocampal LTP induced by a θ -burst protocol (Alarcon et al. 2004). Third, CPEB knockout mice have a deficit in a form of memory known as extinction, which is a behavioral response that will diminish and gradually become extinct in the absence of reinforcement. Several studies make it clear that extinction is an active process that results in the formation of new memories and is not equivalent to forgetting. It also is clear that mechanisms underlying extinction can be distinct from those underlying acquisition and storage of memories (Abel and Lattal 2001). Extinction involves several brain areas including the hippocampus, amygdala, and prefrontal cortex, all of which contain CPEB (Berger-Sweeney et al. 2006). Furthermore, microarray analysis identified several mRNAs whose levels in the hippocampus differed in the CPEB knockout mouse compared to wild type (Berger-Sweeney et al. 2006); such mRNAs could be downstream from CPEB regulation. Finally, CPEB is present in invertebrate neurons as well, and these cells also support cytoplasmic polyadenylation (Liu and Schwartz 2003). In *Aplysia* sensory neurons in which CPEB RNA has been ablated by an antisense oligonucleotide, LTF is not properly maintained (Si et al. 2003b). Collectively, these results suggest that CPEB-mediated translation could result in the establishment of a “tag” at synapses and that the recognition of this tag by neurons is important for synaptic plasticity and, as a consequence, memory consolidation.

The form of CPEB in *Aplysia* and other invertebrate neurons differs from that in vertebrate neurons in that it contains a long stretch of polyglutamine. Polyglutamine is often found in proteins that have characteristics of a prion, i.e., an infectious agent consisting entirely of protein that is self-reproducing. This observation, plus the fact that CPEB RNA is detected in *Aplysia* neurons prompted Si et al. (2003a,b) to suggest that CPEB might assume a prion-like structure following synaptic stimulation, thereby forming an indelible mark at synapses. In such a scenario, CPEB itself might comprise the synaptic tag, rather than mediating the synthesis of a protein at synapses that constitutes the tag. These investigators showed that *Aplysia* CPEB had some features of a prion in vitro, such as resistance to protease and fast sedimentation rate in sucrose gradients. However, the most compelling evidence comes from work with yeast, where Si et al. (2003b) demonstrated that the *Aplysia* CPEB could assume two forms: One that is aggregated (i.e., prion-like) and one that is not. Surprisingly, these investigators showed that the aggregated form of CPEB is the one that binds RNA in vitro and that the aggregated form of the protein can convert the nonaggregated form into an aggregated form. Such epigenetic in-

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heritance is a fundamental hallmark of prion formation. These authors speculate that synaptic stimulation causes CPEB to assume a prion-like form and that such a form either stimulates the translation of some RNAs, causes it to alter its substrate specificity, or releases some mRNAs from an inhibited state. Most importantly, the authors suggest that once in a prion-like form, CPEB needs no further stimulation (i.e., by kinases) to maintain its activity. Beyond doubt, a demonstration that CPEB does indeed form a prion-like structure in neurons would constitute a major advance in neuroscience.

If polyglutamine-containing CPEB forms a prion-like form in invertebrate neurons, then what about the polyglutamine-lacking CPEB in vertebrate neurons? Vertebrates contain three additional CPEB-like genes (Mendez and Richter 2001), all of which are expressed in the brain (Theis et al. 2003). Two of these other CPEB-like proteins do contain polyglutamine, although they are not nearly as long as that in the *Aplysia* CPEB. Whether these other CPEB-like proteins form prion-like structures, or indeed even regulate polyadenylation or translation, is unknown at this time.

FRAGILE X MENTAL RETARDATION SYNDROME AND FMRP

It has been estimated that one in approximately 4000 males and one in about 8000 females has the fragile X syndrome, a heritable form of mental retardation most obviously manifested by mild to severe mental retardation and connective tissue abnormalities. The fragile X gene is one that is subject to expansion by CGG triplets; fewer than 60 produce little evidence of the disease, whereas more than 200 lead to the most severe phenotypes. The repeat expansion occurs in an exon that gives rise to the mRNA 5'UTR (Gatchel and Zoghbi 2005) and the greater the number of repeats, the less FMRP (the protein derived from the fragile X gene) is produced. FMRP is widely expressed in animal tissues and shuttles between nucleus and cytoplasm; in neurons of the central nervous system (CNS), it is found postsynaptically. FMRP is a complex RNA-binding protein containing three KH domains (i.e., has homology with the RNA-binding region of heterogeneous nuclear RNP K) and an RGG (arginine-glycine-glycine) box. These observations suggest that FMRP might regulate local, synaptic mRNA translation and that the loss of this regulation could result in fragile X mental retardation. Perhaps the most compelling evidence that this is the case is the observation that an individual with a point mutation (I304N) in the second KH domain, which disrupts RNA binding in vitro (Siomi et al. 1994), has a severe fragile X phenotype (De Boulle et al. 1993; O'Donnell and Warren 2002; Bagni and Greenough 2005; Darnell et al. 2005).

How does FMRP regulate translation, and is the regulation positive or negative? The answers are not clear-cut, in part because of substantial discordance in the experimental data. First, the seemingly straightforward issue as to whether FMRP associates with polyribosomes is controversial. Some investigators find that the protein sediments with polysomes (Feng et al. 1997; Khandjian et al. 2004; Stefani et al. 2004), whereas others find that it sediments with nontranslating (i.e., slowly sedimenting, $\leq 80s$) RNPs (Siomi et al. 1996; Zalfa et al. 2003), and yet others find that similar amounts are present in both fractions (Brown et al. 2001). Variables such as the type of detergent and/or ionic strength of the solutions used in cell fractionation, source of the biological material, and even age of the animals all contribute to this uncertainty; therefore, a general consensus as to the molecular function of this protein has not yet emerged. Second, several screens to identify mRNA targets of FMRP have yielded minimally overlapping sequence sets, again owing to differences in methodologies and sources of material used for the identification (Brown et al. 1998; Mayashiro et al. 2003).

Two observations suggest that FMRP regulation of translation might involve microRNAs (miRNAs) (for a discussion of miRNAs, see Chapter 11). First, immunoprecipitation of tandem affinity-tagged FMRP from *Drosophila* cells (notated as dFMR1) coimmunoprecipitated argonaute-2, a component of the RISC (RNA-induced silencing complex) (Ishizuka et al. 2002). Second, an analysis of RISC components in *Drosophila* cells revealed the presence of FMRP (Caudy et al. 2002). These observations do not necessarily imply a function for FMRP in the RISC complex, but it is compelling that several laboratories have reported that both FMRP (see above) and miRNAs are associated with polysomes (Olsen and Ambros 1999; Kim et al. 2004; Nelson et al. 2004), although this contention has also been disputed (Pillai et al. 2005). In *Drosophila*, Jin et al. (2004a,b) found that the neuromuscular junction is normal when dFMR-2 or argonaute-1 is heterozygous, but that there is a synaptic overgrowth phenotype when both genes are heterozygous in the same fly. Coupled with the observations that dFMR-1 is a component of RISC, this genetic interaction implies that these two proteins interact functionally and suggests that dFMR-1 might work with miRNAs to control translation.

Although FMRP-associated miRNAs have not yet been identified, another small RNA, unrelated to miRNA or other components of the RNA interference (RNAi) pathway, appears to be involved with FMRP-controlled translation. Zalfa et al. (2003) found that FMRP-mediated translational repression of α CaMKII mRNA at synapses of the mouse brain involves BC1 RNA, a small noncoding RNA long known to be dendritic (Chicurel et al. 1993). BC1 not only interacts with FMRP, but base-pairs

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with α CaMKII RNA, leading to the postulate that BC1-containing duplexes are promoted by FMRP (Bagni and Greenough 2005). How such an activity might cause translational repression is unknown. In contrast to these results, however, Wang et al. (2005) could detect no significant BC1 RNA–FMRP interaction. Rather, these authors found that BC1 RNA inhibits 48S initiation complex formation through associations with both eIF4A and poly(A)-binding protein. Under these circumstances, BC1 would probably act as a general inhibitor of translation, rather than an mRNA-specific inhibitor as suggested by Zalfa et al. (2003).

In summary, although most investigators in the fragile X field agree that one function of FMRP is to repress translation, the mechanism by which it does so has not been elucidated. On the other hand, that FMRP has a critical role in synaptic plasticity is seemingly beyond doubt. Huber et al. (2002) demonstrated that FMRP knockout mice have an enhanced mGluR-LTD, which, as noted previously, is protein-synthesis-dependent. How important are mGluRs in fragile X? Very important, at least as far as fruit flies are concerned. Mutant flies lacking the *dfmr1* gene have abnormal courtship behavior, poor memory, and structural anomalies in a part of the brain known as the mushroom bodies (Zhang et al. 2001; Dockendorff et al. 2002; Morales et al. 2002; Lee et al. 2003). The ingestion of drugs that antagonize mGluR signaling in mammals rescues all of these phenotypes (McBride et al. 2005)! Clearly, such results offer possible therapies for treating human fragile X mental retardation (Bear 2005; Dolen and Bear 2005).

PERSPECTIVES

One intriguing insight from the studies of translational control in synaptic plasticity conducted thus far is that the basic signal transduction cascades that are required to initiate translation are similar, regardless of whether the plasticity induced results in either LTP or LTD. In some ways, this makes sense. To synthesize the new proteins required for the expression of either LTP or LTD, translation must be initiated. Kelleher et al. (2004a) have suggested that long-lasting forms of LTP and LTD elicit a global regulation of protein synthesis via stimulation of these core signal transduction cascades that initiate translation. How then do LTP-specific and LTD-specific proteins get synthesized? As discussed earlier, one possibility is gene-specific regulation via mRNA-binding proteins such as CPEB and FMRP. Interestingly, CPEB (Huang et al. 2003) and FMRP (Rackham and Brown 2004; Antar et al. 2005), as well as the double-stranded RNA-binding protein staufen (Kiebler et al. 1999; Tang et al.

2001; Kanai et al. 2004), are all involved in RNA transport in dendrites. How these proteins transport mRNAs (presumably untranslated) is of considerable interest and may involve a large number of factors including molecular motors (Krichevsky and Kosik 2001; Kanai et al. 2004). A thorough review of RNA transport in neurons has been published recently (Kindler et al. 2005).

Whether either LTP or LTD is ultimately expressed must be due to either a difference in the synthesis of a small number of specific proteins or the creation of specific synaptic tags, one for LTP and another for LTD, that would permit the "capture" of distinct proteins from a large, homogeneous pool of newly synthesized plasticity proteins (Kelleher et al. 2004a). If the latter is true, one would predict that LTP and LTD would induce the synthesis of a similar pool of proteins that would be sufficient to enable the opposite type of plasticity (i.e., LTP would enable LTD, and LTD would enable LTP). In fact, this type of heterosynaptic associativity between LTP and LTD has been demonstrated (Sajikumar and Frey 2004). The idea that LTP and LTD result in the synthesis of similar sets of proteins remains to be determined, perhaps with either microarray studies of polysome fractions or proteomic analysis.

Reviews recount the past, and thus by their nature are a recitation of facts, or what pass for facts until superseded by new results that can destroy even central dogmas; imagining the future is the challenge. One now established "fact" is that local protein synthesis is required for synaptic plasticity and that modifiers of mRNA translation in neurons such as 4E-BP, CPEB, and FMRP are regulators of this process. What remains a black box, as mentioned above, are the identities of the proteins that are synthesized locally *and* that are modifiers of synaptic efficacy. Although several avenues of investigation will no doubt be necessary to reveal the full panoply of molecules involved, one intriguing route has recently been taken by Schratt et al. (2006), who demonstrate that a specific miRNA, mir-134, is not only localized to the synaptodendritic compartment, but that its regulation of Lim1 kinase (Limk1) mRNA expression controls synaptic spine development. Limk1 regulates the actin cytoskeleton by phosphorylating and inactivating cofilin, an actin-depolymerizing molecule; Limk1 knockout mice display enhanced hippocampal LTP (Meng et al. 2002). Given that there are nearly 100 miRNAs in neurons (Kim et al. 2004) that are regulated throughout development (Krichevsky et al. 2003), it seems reasonable to speculate that they will be found to be important modulators of local translation and synaptic plasticity. Moreover, the identities of the mRNAs to which the miRNAs anneal will help establish the molecular framework of tagging and plasticity, and learning and memory as well.

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