W.S. Sossin, J.-C. Lacaille, V.F. Castellucci & S. Belleville (Eds.)
Progress in Brain Research, Vol. 169
ISSN 0079-6123

CHAPTER 5

Translational control of gene expression: a molecular switch for memory storage

Mauro Costa-Mattioli and Nahum Sonenberg*

Department of Biochemistry and McGill Cancer Center, 3655 Promendde Sir William Osler, McGill University, Montréal, QC H3G 1Y6, Canada

Abstract: A critical requirement for the conversion of the labile short-term memory (STM) into the consolidated long-term memory (LTM) is new gene expression (new mRNAs and protein synthesis). The first clues to the molecular mechanisms of the switch from short-term to LTM emerged from studies on protein synthesis in different species. Initially, it was shown that LTM can be distinguished from STM by its susceptibility to protein synthesis inhibitors. Later, it was found that long-lasting synaptic changes, which are believed to be a key cellular mechanism by which information is stored, are also dependent on new protein synthesis. Although the role of protein synthesis in memory was reported more than 40 years ago, recent molecular, genetic, and biochemical studies have provided fresh insights into the molecular mechanisms underlying these processes. In this chapter, we provide an overview of the role of translational control by the eIF2α signaling pathway in long-term synaptic plasticity and memory consolidation.

Keywords: translational control; synaptic plasticity; long-term memory; GCN2; eIF2α

Overview of translation initiation in eukaryotes

Translational control is an important mechanism by which cells govern gene expression, providing a rapid response by the cell without invoking nuclear pathways for mRNA synthesis and transport. In systems with little or no transcriptional control (e.g., reticulocytes, sea urchin eggs, *Drosophila* early embryogenesis, and oocytes), translation is the major mode of regulation of gene expression (Mathews et al., 2007a). Initiation is the rate-limiting step of translation and the main target of control. This regulation primarily involves the reversible phosphorylation of key eukaryotic initiation factors (eIFs). Phosphorylation of several initiation factors (eIF2B, eIF3, eIF4B, eIF4E, eIF4G) positively correlates with increased translation rates, whereas phosphorylation of other eIFs (e.g., eIF2α) results in inhibition of translation and suppression of cell growth (Raught and Gingras, 2007). The fundamentals and regulatory mechanisms of eukaryotic protein synthesis have been reviewed in a recent book by Mathews et al. (2007b). The key events in initiation are: (i) formation of the 43S ribosomal preinitiation complex, (ii) binding of the mRNA to the 43S complex, (iii) a start codon selection, (iv) 80S complex formation, and (v) recycling of eIF2 to generate a new ternary complex [eIF2 · Met − $tRNA_i^{Met}$ · GTP] (Fig. 1).

Ribosome recruitment to the mRNA is mediated by the eIF3 and eIF4 group of eIFs and occurs by one of two mechanisms: a cap-dependent

*Corresponding author. Tel.: +1 (514) 398-7274/5;
Fax: +1 (514) 398-1287; E-mail: nahum.sonenberg@mcgill.ca

DOI: 10.1016/S0079-6123(07)00005-2

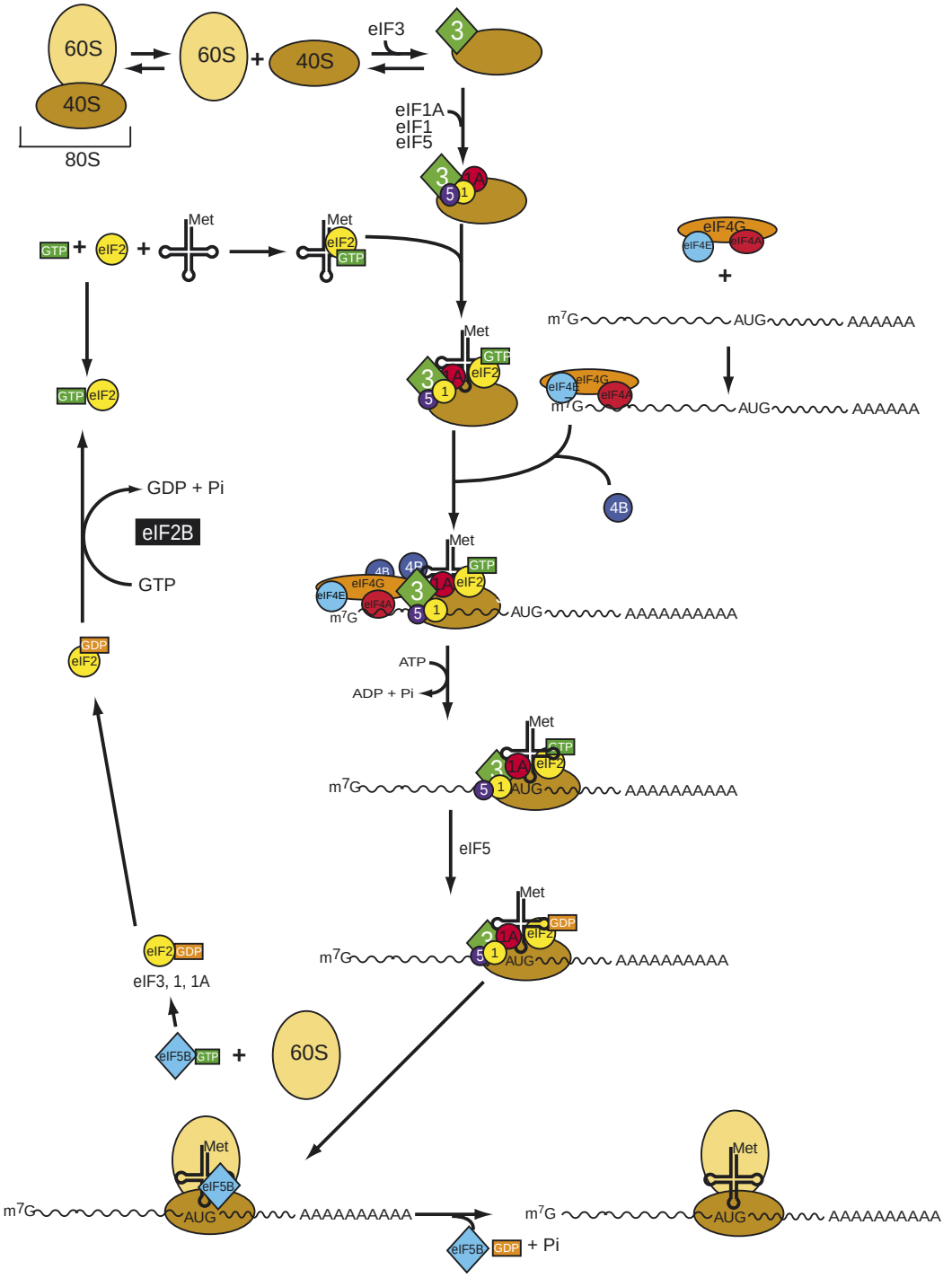

Fig. 1. Schematic representation of the translation initiation pathway in eukaryotes. Eukaryotic translation initiation factors are indicated as color-coded circles. (See Color Plate 5.1 in color plate section.)

(Pestova et al., 2007, p. 887) or a cap-independent mechanism, the latter involving internal recruitment of the ribosome to the mRNA 5′ UTR (Doudna and Sarnow, 2007, p. 864; Elroy-Stein and Merrick, 2007, p. 863). In eukaryotes, initiation on most mRNAs is thought to occur via a cap-dependent process. Indeed, the 5′ cap structure (m7GpppN, where m is a methyl group and N is any nucleotide), which is present at the 5′ end of all nuclear-transcribed eukaryotic mRNAs, is the first mRNA structure that is recognized by eIFs (Fig. 1). The protein complex involved in this step is eIF4F, which consists of: (i) eIF4E, the cap-binding protein responsible for directing eIF4F to the mRNA cap structure; (ii) eIF4A, an RNA helicase required to unwind local secondary structure to facilitate access of the 43S ribosomal complex to the mRNA template; and (iii) eIF4GI or eIF4GII (encoded by two different genes), modular scaffolding proteins that bridge the mRNA to the ribosome through interactions with eIF3, the largest and most complex eIF. The mammalian eIF3 contains 13 subunits that are designated eIF3a to eIF3m (Hinnebusch, 2006). In contrast, the yeast eIF3 version contains orthologs of only five mammalian eIF3 subunits eIF3a, eIF3b, eIF3c, eIF3g, and eIF3j. These five subunits are considered the core eIF3. eIF3 interacts with the 40S ribosomal subunit, thus serving as a link between the mRNA–eIF4F complex and the ribosome (Pestova et al., 2007).

Once bound to the mRNA, the 43S complex is thought to scan the 5′ UTR, supported by ATP hydrolysis, until the appropriate AUG start codon is encountered (Fig. 1). Because eIF4E is the least abundant of all the initiation factors, the mRNA recruitment step is rate-limiting (Duncan et al., 1987).

Regulation of translation initiation

Translational control of protein synthesis is generally achieved by changes in the phosphorylation state of eIFs or their regulators (see below). Two main targets for regulation are (i) the phosphorylation of eIF2α that regulates the exchange of GDP for GTP on eIF2 and (ii) the formation of eIF4F that controls the recruitment of the mRNA to the ribosome. Our review will focus on the translational control by the eIF2α signaling pathway. For a more detailed review on the role of the eIF4F complex and its regulators, eIF4E-binding proteins (4E-BPs) in translational control of long-lasting plasticity and learning and memory, see Chapter 10.

Translation regulation by phosphorylation of eIF2α

Translational control by phosphorylation of eIF2α is one of the best characterized translational mechanisms in eukaryotic cells. eIF2 consists of three subunits: a large γ subunit and two smaller α and β subunits. eIF2 binds both GTP and the $\mathrm{Met}-\mathrm{tRNA}_{\mathrm{i}}^{\mathrm{Met}}$ to form a ternary complex. eIF2 associates with the small ribosomal subunit in its GTP-bound form. Like other GTP-binding proteins, eIF2 alternates between its GTP-bound state and its GDP-bound state. GTP is hydrolyzed when the initiator AUG is engaged by the ribosome to produce eIF2 in the GDP-bound state. Exchange of GDP for GTP on eIF2 is catalyzed by the pentameric guanine nucleotide exchange factor eIF2B, and is required to reconstitute a functional ternary complex for a new round of translation initiation (Hinnebusch et al., 2007; Pestova et al., 2007). eIF2α is critical for the modulation of eIF2's activity. Phosphorylation of the α subunit on Ser51 decreases general translation initiation (Dever, 2002) by blocking the GDP–GTP exchange reaction and reducing the dissociation rate of eIF2 from eIF2B (Fig. 2). Since the cellular levels of eIF2B are much lower than the levels of eIF2, even when only a fraction (about 20–30%) of eIF2α is phosphorylated and complexed with eIF2B, the GTP–GDP exchange process is inhibited. Paradoxically this also results in stimulation of translation of a subset of mRNAs that contain upstream open reading frames (uORFs) (Dever et al., 2007; Hinnebusch et al., 2007; Jackson et al., 2007) (Fig. 2).

The molecular mechanism underlying this selective translation was extensively studied in the general amino acid control response in the yeast *S. cerevisiae* (Hinnebusch et al., 2007). Amino acid starvation of yeast cells increases translation of GCN4 mRNA, a process strongly dependent on the activation of the eIF2α kinase Gcn2p and

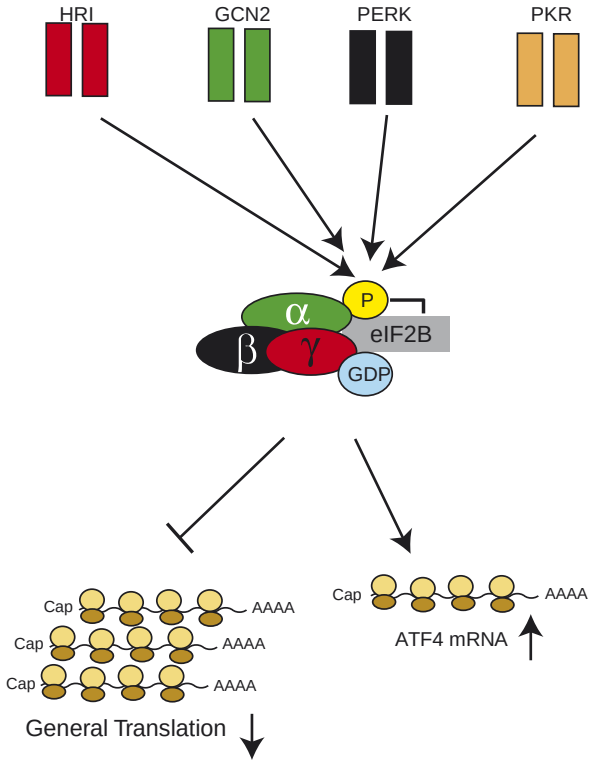

Fig. 2. Schematic representation of the eIF2α signaling pathway. The four eIF2α kinases (GCN2, PERK, PKR, and HRI), whose activity is regulated by different stress signals, phosphorylate Ser51 on the α subunit of eIF2. Phosphoryation of eIF2α leads to inhibition of general translation but it stimulates translation of ATF4 mRNA. (See Color Plate 5.2 in color plate section.)

phosphorylation of eIF2α. The specific induction of GCN4 translation upon eIF2α phosphorylation is mediated by four short open reading frames in the leader of GCN4 mRNA. When amino acids are available, scanning ribosomes translate these short ORFs but dissociate from the mRNA before reaching the authentic GCN4 start codon. In contrast, under starvation conditions, eIF2α phosphorylation by GCN2 inhibits eIF2B, thus causing a fraction of the scanning 40S subunits to form active translational complexes only after they bypassed the upstream ORFs, and allowing initiation at the proper GCN4 start codon (Hinnebusch et al., 2004, 2007).

In mammals, the translation of the Gcn4's metazoan counterpart, ATF4 (CREB2), is enhanced by eIF2α phosphorylation (Harding et al., 2000; Lu et al., 2004; Vattem and Wek, 2004). ATF4 contains two uORFs which are highly conserved across species. Both uORFs contribute in a different manner to ATF4 mRNA translation. The 5′-proximal uORF1, which is shorter than uORF2, is a positive element required to enhance translation of ATF4 mRNA in response to high eIF2α phosphorylation levels. In contrast, uORF2 overlaps the ATF4 ORF, rendering ribosomes that translate it unable to access the authentic ATF4 initiation site (Fig. 3). Similar to GCN4 mRNA translation, lowering the concentration of ternary complex (by phosphorylation of eIF2α) increases the probability that any rescanning 40S subunit will acquire the ternary complex at the ATF4 initiation site (Lu et al., 2004; Vattem and Wek, 2004).

eIF2α kinases

In mammalian cells, there are four known Ser/Thr protein kinases for which eIF2α is the major

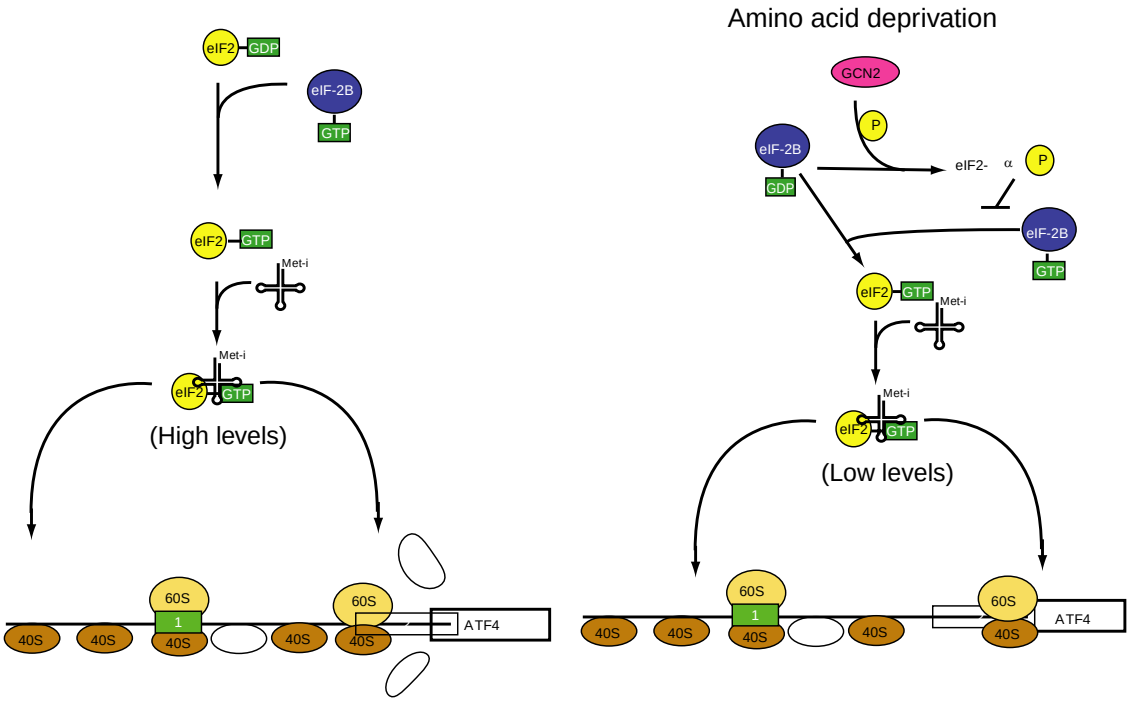

Fig. 3. A model for ATF4 mRNA translation. Schematic diagram of the 5′ untranslated region of ATF4 mRNA. The open reading frames (ORFs) are shown as green boxes, and the ATF4 mRNA authentic ORF as an open rectangle. Under normal growing conditions (left panel) the 40S ribosome initiates at ORF1 and reinitiates at ORF2. Under amino acid deprivation conditions (right panel), due to a low concentration of ternary complex, 40S ribosomes conditions failed to reinitiate at ORF2 but reinitiated instead at the authentic ORF. (See Color Plate 5.3 in color plate section.)

substrate. They include the double-stranded (ds) RNA-activated protein kinase (PKR), the hemin-regulated inhibitor kinase (HRI), the pancreatic eIF2α or the PKR-endoplasmic reticulum (ER)-related kinase (PEK/PERK), and the general control non-derepressible kinase (GCN2). The eIF2α kinases share a conserved kinase domain (Dever et al., 2007) and their ability to respond to different stimuli is due to the presence of regulatory domains. For instance, double-stranded RNA (dsRNA), heme deficiency, misfolded proteins in the ER, and amino acid deprivation activate PKR, HRI, PERK, and GCN2, respectively.

With the exception of HRI whose level of expression is very low, all the eIF2α kinases are significantly expressed in the brain (Meurs et al., 1990; Chen et al., 1991; Chong et al., 1992; Crosby et al., 1994; Mellor et al., 1994; Shi et al., 1998; Berlanga et al., 1999; Harding et al., 1999; Sood et al., 2000). We will describe the most salient aspects of GCN2 since it plays a critical role in synaptic transmission, learning, and memory. GCN2 is the ancestral eIF2α kinase and is present in all eukaryotes from yeast to mammals (Hinnebusch et al., 2004; Dever et al., 2007).

GCN2 is activated under conditions of amino acid deprivation via the accumulation of uncharged tRNA. GCN2 has five domains. At the N-terminal, it contains a charged region which binds GCN1 and is required in vivo for activation of the kinase domain. This domain is followed by a pseudokinase domain, the eIF2α kinase domain, a domain related to histydyl-tRNA synthetase (HisRS), which includes a sequence (motif 2) interacting with all the deacylated tRNAs with similar affinity, and a carboxy-terminal domain which enhances tRNA binding, dimerizes, and mediates binding to ribosomes. In contrast to

PKR and PERK, which are monomers and their kinase activation requires dimerization, GCN2 is thought to be a constitutive dimer. In addition, the HisRS interacts with both the kinase domain and the carboxy-terminal domain. These inter-domain interactions are believed to keep the kinase in an inactive state. It is believed that the uncharged tRNAs, which increase in response to amino acid deprivation, bind to HisRS domain and cause the release of these inhibitory inter-domains interacting, thus activating the kinase.

GCN2 is also activated by other stresses such as UV irradiation, high salinity, glucose limitation, and rapamycin (Deng et al., 2002; Narasimhan et al., 2004). Interestingly, these stresses cannot activate a mutant GCN2 which lacks the m2 motifs, indicating that uncharged tRNA must be the main activator of GCN2.

Pharmacologic evidence that translation regulates long-term synaptic plasticity and memory

Short-term and long-term memory

The idea about two memory systems (STM and LTM) has emerged from the study of patients with memory impairments. A classic in the medical literature is the case of a patient, H.M., who suffered from seizures due to a head injury in a bicycle accident when he was 9 years-old (Scoville and Milner, 1957). To relieve his intractable seizures, neurosurgeons performed a bilateral surgical excision of the medial temporal region. As a result of the surgery H.M. exhibited a severe impairment in LTM but STM was intact. Further studies on other species have supported the same distinction between STM and LTM (Scoville and Milner, 1957).

Protein synthesis and behavioral learning

A similar idea about STM and LTM has emerged from the study of protein synthesis inhibitors (puromycin, anysomicin, emetine, acetoxycycloheximide, cycloheximide, and rapamycin) in memory formation. These studies provided the first molecular clues about the distinction between these two processes. The initial studies by Flexner et al. (1963), Agranoff et al. (Agranoff and Klinger, 1964), and Squire and Barondes (Davis and Squire, 1984), which showed that protein synthesis inhibitors block declarative memory, revolutionized the memory and cognition field. Further behavioral studies in different species strengthened the notion that protein synthesis inhibitors block specifically long-term memory (LTM) formation whereas short-term memory (STM) is spared. Interestingly, protein synthesis inhibitors are effective when given immediately before or after training. Indeed, when they are applied 1 h or later after training LTM is not affected (Davis and Squire, 1984).

Studies on the gill-withdrawal reflex of the marine mollusk *Aplysia californica* revealed similar results. A single stimulus to the tail gives rise to a protein synthesis independent, short-lasting sensitization (minutes to hours). In contrast, repetition of such a stimulus elicits LTM sensitization that can last days to week and requires the synthesis of new proteins (Pinsker et al., 1973; Carew et al., 1983; Frost et al., 1985; Castellucci et al., 1989). Taken together these data indicate that protein synthesis during or shortly after training is required for long-term but not STM.

Protein synthesis and long-lasting plasticity

Repeated activity strengthens synaptic connections between brain cells. This process, known as long-term potentiation (LTP), is believed to be a key cellular mechanism by which information is stored (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999; Kandel, 2001). The first evidence that new protein synthesis is required for long-lasting LTP came from in vivo LTP experiments (Krug et al., 1984). Further studies on hippocampal slices in vitro have shown that two distinguished temporal phases of LTP were based on the sensitivity to protein synthesis inhibitors (Kelleher et al., 2004; Klann and Dever, 2004). Like memory, LTP exhibits two temporally distinct phases: early LTP (E-LTP), which depends on modification of pre-existing proteins; and late LTP (L-LTP), which requires transcription and synthesis of new proteins (Silva et al., 1998; Kelleher et al., 2004; Klann

and Dever, 2004; Sutton and Schuman, 2006; Costa-Mattioli et al., 2007b). E-LTP is typically induced by a single train of high-frequency (tetanic) stimulation 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 lasts many hours.

Consistent with these data, Montarolo and colleagues (Montarolo et al., 1986) showed that in *Aplysia* sensory neurons protein synthesis inhibitors blocked serotonin-mediated long-term facilitation but not short-term facilitation. In parallel to the behavior and plasticity experiment in vertebrates, the protein synthesis inhibitors were only effective if given around the time of the serotonin application.

At the molecular level, co-activation of pre- and post-synaptic neurons leads to the transcription of plasticity-related genes whose mRNAs are either subsequently translated in the cell body or transported to synapses on the dendrites where they are locally translated. The newly synthesized proteins are somehow captured by specifically ''tagged'' synapses, those most recently active (Frey and Morris, 1997; Martin et al., 1997). This is inferred from the finding that strongly stimulated synapses can enable weakly stimulated ones to generate L-LTP (reviewed by Morris and Frey, 1999; Martin and Kosik, 2002). Translation is also regulated locally (independently of changes in gene transcription) at stimulated synapses. The local synthesis model is supported by the presence of ribosomes and mRNAs in, or close to, dendritic spines. This process has been extensively reviewed (Steward and Schuman, 2001, 2003; Sutton and Schuman, 2006).

Genetic evidence that translation regulates long-term synaptic plasticity and memory

Identification of GCN2 as regulator of learning and memory and characterization of GCN2 knockout mice

Though we knew that memory consolidation requires new protein synthesis, the molecular mechanisms by which translation controls these processes remained obscure. GCN2 has several interesting features: GCN2-mediated phosphorylation of eIF2α suppresses general translation and selectively stimulates the translation of ATF4 (Dever et al., 2007; Pestova et al., 2007; Ron and Harding, 2007). Interestingly, ATF4 and its homologs are repressors of long-lasting synaptic plasticity and memory formation in diverse phyla (Bartsch et al., 1995; Abel et al., 1998; Kandel, 2001; Chen et al., 2003). More importantly, GCN2 is the major eIF2 kinase in the brain. Its mRNA is enriched in the brain of flies (Santoyo et al., 1997) and mammals (Berlanga et al., 1999; Sood et al., 2000), especially in the hippocampus (Costa-Mattioli et al., 2005). Thus, the well-documented requirement for translation in modulating synaptic activity and memory, together with strong evidence linking eIF2α phosphorylation and translational control to ATF4 activity (Lu et al., 2004; Vattem and Wek, 2004), raised the intriguing possibility of a role for GCN2 in regulating synaptic plasticity, learning, and memory. To explore this possibility, a GCN2 knockout mouse was generated. The GCN2 gene was mutated by deleting the essential exon 12 (Costa-Mattioli et al., 2005). In addition, splicing of exon 11 to exon 13 was predicted to disrupt the reading frame of the mRNA and introduce multiple stop codons which destabilize the mRNA. GCN2 was absent in the hippocampus of GCN2 knockouts as determined by two different antibodies which recognize the amino and carboxy-terminal of the protein. The GCN2 knockout mice are viable, fertile, and develop normally. In the hippocampus of GCN2 knockouts, both eIF2α phosphorylation and the memory repressor ATF4 are reduced (Costa-Mattioli et al., 2005).

We first examined synaptic plasticity at synapses on CA1 pyramidal cells by recording in stratum radiatum extracellular excitatory postsynaptic potentials evoked by electrical stimulation of the Schaffer collateral pathway. Basal synaptic transmission (i.e., input–output curves, fiber volley amplitude, and pair pulse facilitation) was not altered in GCN2 knockout mice. Surprisingly, a protocol that usually elicits an E-LTP, which is independent of gene expression (translation and

transcription), elicited a typical gene expression-dependent L-LTP in hippocampal slices from GCN2 knockout mice (Costa-Mattioli et al., 2005). By three independent criteria, the LTP generated in GCN2 knockout mice is indistinguishable from the normal L-LTP induced by four trains of stimulation: it is (i) dependent on gene expression, (ii) dependent on PKA, and (iii) immune to depotentiation. The ability to convert a stimulus that normally leads to a short-term change to a long-term change in plasticity has been seen in numerous genetic manipulations in several species (Bartsch et al., 1995; Yin et al., 1995; Malleret et al., 2001; Barco et al., 2002; Genoux et al., 2002; Chen et al., 2003), and is invariably due to a decrease in the threshold for activating gene expression. For instance, mice with enhanced CREB activity (Barco et al., 2002) or those expressing a dominant negative for ATF4 (Chen et al., 2003) are remarkable examples of this principle: as in the GCN2 knockout mice, a single train of high-frequency stimulation was sufficient to elicit a sustained L-LTP in these mice. In this regard, it is noteworthy that in the basal state, GCN2 knockout mice exhibit decreased ATF4 levels. Thus, a plausible interpretation is that the facilitated LTP elicited in GCN2 knockout mice is associated with a lower threshold for activation of gene expression. Therefore, the effect of GCN2 on long-lasting changes in plasticity could be mediated through modulating ATF4/CREB activity. However, to our surprise, a L-LTP inducing protocol, such as four trains at 100 Hz or forskokin, elicited an impaired L-LTP in the GCN2 knockout hippocampal slices.

Deleting GCN2 affects LTP but not long-term depression (LTD), another well-characterized form of synaptic plasticity, induced by either low-frequency stimulation (LFS) or incubation with DHPG, an agonist of group I mGluRs (Costa-Mattioli et al., 2005). Therefore, it is possible that GCN2 does not play any role in protein synthesis-dependent LTD or the signaling pathway which regulates GCN2 activity is not altered by LTD-inducing protocols.

Is the altered synaptic efficacy in the hippocampal neurons of GCN2 knockout mice manifested at the behavioral level? To answer this question, mice were first subjected to Pavlovian fear conditioning. Pairing tone presentations with a foot shock in a particular environment leads to both auditory and contextual fear conditioning. Contextual fear conditioning is a hippocampus-dependent process in which LTM for the context is established following a single training session. Auditory fear conditioning on the other hand, which associates a tone (CS) with the foot shock (US), is dependent on the amygdala but not the hippocampus (Fanselow and LeDoux, 1999; LeDoux, 2000). Both contextual and cue fear conditioning are dependent on new protein synthesis (Bourtchouladze et al., 1998; Schafe et al., 1999).

GCN2 knockout mice exhibited reduced "freezing" when tested after a retention delay of 24 h and 10 days after training, indicating that hippocampus-dependent fear memories are impaired in these mice. By contrast, when GCN2 knockout mice were evaluated in auditory fear conditioning, they exhibited normal associative memory for the tone. These data indicate that the lack of GCN2 selectively affects hippocampal-dependent memories but not amygdala-dependent auditory fear conditioning.

To further assess the role of GCN2 in hippocampal-dependent memories, wild-type (WT) and GCN2 knockout mice were subjected to the hidden-platform version of the Morris water maze, a hippocampus-dependent reference memory task, in which a mouse must find a platform (using visual spatial clues) hidden under opaque water in a pool (Morris et al., 1982). Using a conventional training protocol (three trials per day), spatial learning of GCN2 knockout mice was impaired, as determined by escape latencies and quadrant occupancy (Costa-Mattioli et al., 2005). Since a short-lasting LTP protocol induced a robust gene-expression-dependent LTP in GCN2 knockout, mice were trained in a weak training protocol (once per day). Remarkably, when the GCN2 knockout mice were given a weak training, they exhibited enhanced spatial learning and memory (Costa-Mattioli et al., 2005). In parallel to the electrophysiological findings in hippocampal slices of GCN2 knockout mice, where weak stimulation elicited L-LTP instead of E-LTP, but strong

stimulation failed to evoke the expected L-LTP, a weak training protocol induced an enhanced memory but a stronger protocol impaired memory consolidation in these mice. These data provide the first genetic evidence that translational control is critical for long-lasting synaptic plasticity and memory consolidation.

GCN2 in the brain regulates selection of balanced diet

Omnivorous animals such as rats reject diets lacking essential amino acids. Selection of such a balanced diet plays an important role in human evolution. It has been reported that neurons of the apical periform cortex, which project to appropriate feeding neuronal circuits, are activated by intracellular indispensable amino acids (Haberly and Price, 1978). The apical periform cortex appears to be critical for such an adversive response, because bilateral lesion of this region abolishes the bias against diets lacking essential amino acids (Gietzen, 1993). Interestingly, consumption of an imbalanced diet increases the phosphorylation of eIF2α in neurons of the apical periform cortex (Gietzen et al., 2004). Recently, two independent groups showed that GCN2 is responsible for this basic mechanism of functional stress (Hao et al., 2005; Maurin et al., 2005). Both groups showed that WT mice reject diet lacking threonine and leucine whereas GCN2 knockout mice consumed equal amount of balanced and imbalanced diet.

Consistent with these data, injection of amino alcohol threoninol, an inhibitor of a single tRNA synthetase into the apical periform cortex caused a decrease in food intake of a balanced diet (Hao et al., 2005). These data indicated that uncharged tRNA, which as amino acid deprivation activates GCN2, is the signal that triggers the feeding response. Thus, GCN2 appears to be responsible for the increased phosphorylation of eIF2α in neurons of the apical periform cortex. An increase in eIF2α phosphorylation was observed in brain section from WT mice feed with an imbalanced diet whereas the same diet did not alter eIF2α phosphorylation levels in GCN2 knockout brain slices (Hao et al., 2005; Maurin et al., 2005). It will be interesting to learn whether in WT mice either fed with a diet lacking amino acids or injected with amino alcohol threoninol in WT mice, the activity of GCN2 (phosphorylation) also is up-regulated in the apical periform cortex. Indeed, consumption of a diet lacking indispensable amino acids led to an eIF2α phosphorylation in the liver (Anthony et al., 2004; Maurin et al., 2005).

Taken together, these data indicate that GCN2 senses the imbalance in amino acids and activates down-stream signaling pathways which lead to the behavioral rejection of a diet lacking amino acids.

Important question remains with respect to the role of GCN2-mediated eIF2α in sensing amino acid deficiency in mammalian periform cortex. Is ATF4 the downstream target responsible for this or the decrease in translation associated with an increased eIF2α phosphorylation? Does local activation of the other eIF2α kinases, such as PERK or PKR, in the apical periform cortex, recapitulates the phenotype observed in WT fed with a diet lacking amino acids? Finally, it will be interesting to investigate whether other protein kinases also contribute to this feeding behavior.

A master switch for the conversion from short-term to long-term synaptic plasticity and memory formation

Consolidation of long-term memories requires the expression of new genes (Squire, 1987). Thus, if new gene expression is the rate-limiting step necessary to strengthen existing synaptic connections between neurons, how is this process turned on? If one could find such a mechanism and switch it on, then stimulation that normally elicits only E-LTP and STM should lead to L-LTP and LTM. This was the goal of our research. In diverse phyla, a key component in memory formation is the transcription factor CREB (cAMP responsive element-binding protein). CREB is regulated by phosphorylation of its serine 133, and is also under the control of the repressor protein ATF4 (CREB-2) (Bartsch et al., 1995; Silva et al., 1998; Kandel, 2001; Chen et al., 2003), which in turn is regulated at the level of mRNA translation. As described above, phosphorylation of eIF2α

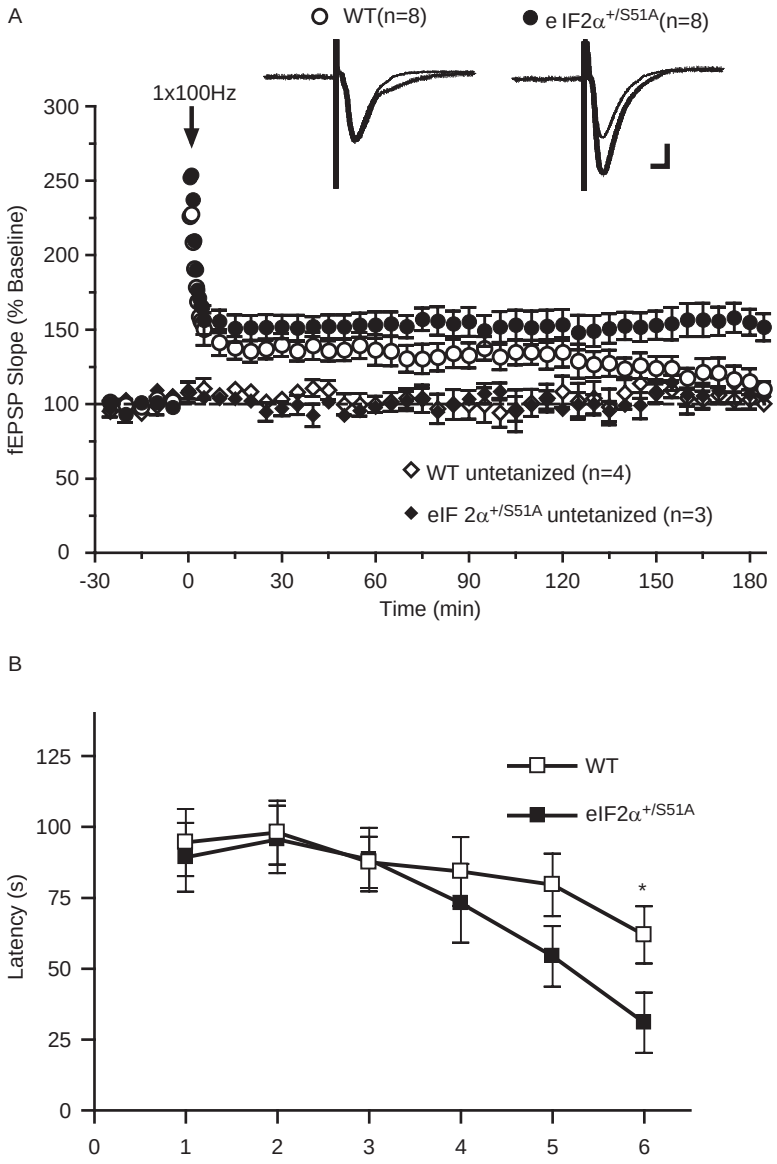

Fig. 4. Decreased eIF2α phosphorylation facilitates L-LTP and long-term spatial memory consolidation. (A) A single train of high-frequency stimulation elicits an enhanced LTP in hippocampal slices from eIF2α$^{+/S51A}$ mice. (B) eIF2α$^{+/S51A}$ mice exhibited and enhanced long-term spatial memory with a weak training protocol in the Morris Water Maze.

suppresses general translation, and selectively stimulates the translation of ATF4 (Dever et al., 2007; Ron and Harding, 2007). Thus, eIF2α phosphorylation regulates two fundamental processes essential for the conversion from short-term to long-term synaptic plasticity and memory: (a) de novo protein synthesis and (b) CREB-mediated gene expression via ATF4. According to recent evidence, eIF2α phosphorylation is tightly correlated with neuronal activity. We and others have shown that L-LTP-inducing protocols decrease eIF2α phosphorylation (Takei et al., 2001; Costa-Mattioli et al., 2005). In keeping with a role in memory formation, eIF2α phosphorylation is reduced when rats are trained in

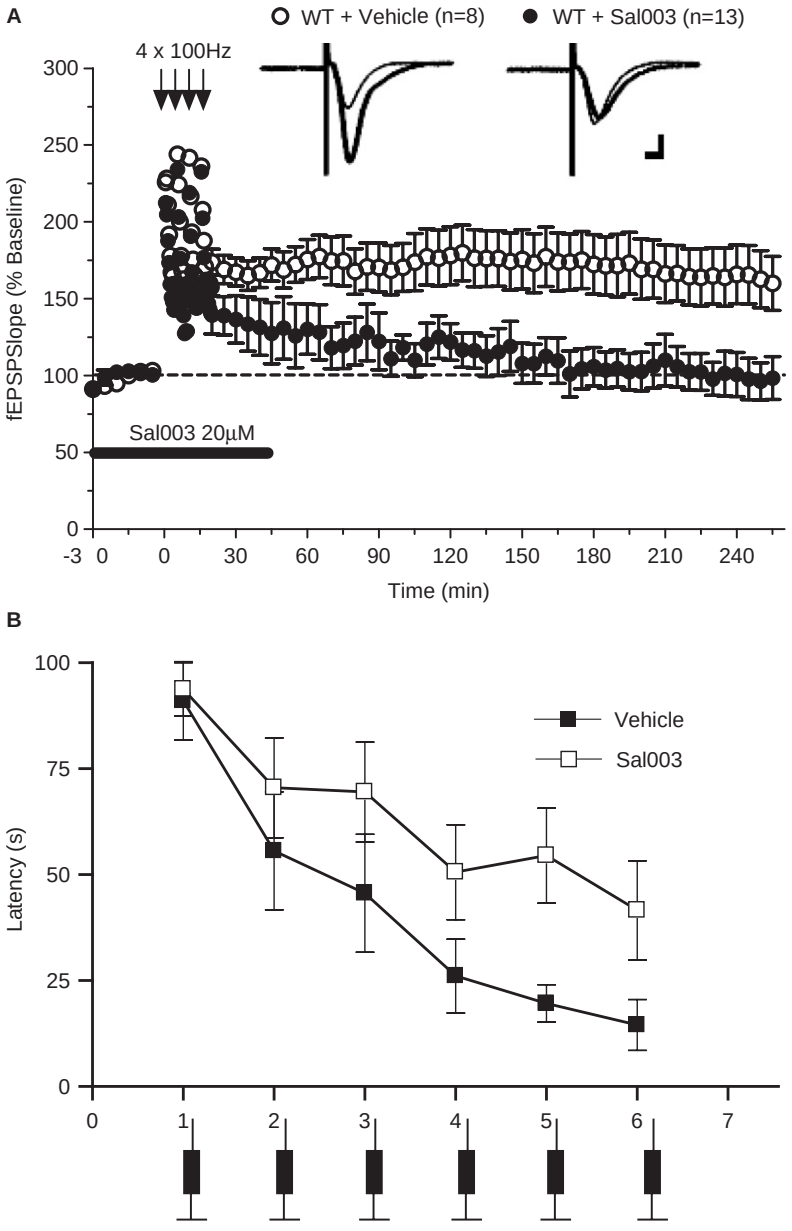

Fig. 5. Increased eIF2α phosphorylation impairs L-LTP and spatial memory consolidation. (A) The induction of L-LTP is blocked by Sal003, an inhibitor of the eIF2α phosphatases. (B) Infusion of Sal003 into the hippocampus immediately after training blocks memory consolidation. Dark syringes refer to either vehicle or Sal003 infusions across groups.

a Pavlovian-fear conditioning task (Costa-Mattioli et al., 2007b). In addition, in mice lacking GCN2 (the main eIF2α kinase in the mammalian brain) eIF2α levels are reduced and synaptic plasticity and memory are altered (Costa-Mattioli et al., 2005). We therefore predicted that reduced eIF2α phosphorylation would facilitate gene expression, L-LTP induction, and LTM storage.

We investigated long-lasting synaptic plasticity and memory in eIF2α heterozygous mutant mice ($eIF2\alpha^{+/S51A}$) in which eIF2α phosphorylation and ATF4 levels are decreased. As expected, LTP was elicited more readily in hippocampal slices from these mice: stimulation that normally induces a short-lasting E-LTP in WT mice elicited a sustained, gene expression-dependent L-LTP in hippocampal slices from $eIF2\alpha^{+/S51A}$ mice (Fig. 4A) (Costa-Mattioli et al., 2007a). Furthermore, mice lacking GCN2, in which eIF2α phosphorylation is reduced (Costa-Mattioli et al., 2005), and those expressing an inducible inhibitor of ATF4 (Chen et al., 2003) had similar phenotypes. In agreement with the enhanced LTP in hippocampal slices, $eIF2\alpha^{+/S51A}$ mice have an enhanced memory and lower threshold for learning in several behavioral tasks, such as the Morris water maze (Fig. 4B), associative fear conditioning, and conditioned taste aversion. These data strongly support the notion that reduced phosphorylation of eIF2α facilitates the expression of genes required for long-lasting synaptic plasticity and memory consolidation.

We reasoned that, if dephosphorylation of eIF2α is essential for memory consolidation, preventing the decrease in eIF2α phosphorylation that normally occurs during memory formation should inhibit not only gene expression but also L-LTP and LTM. To test this prediction, we applied Sal003, a compound which suppresses eIF2α dephosphorylation (Boyce et al., 2005). As expected, the Sal003-mediated increase in eIF2α phosphorylation resulted in inhibition of general translation and selective increase in translation of ATF4 mRNA. Furthermore repeated tetanic stimulation induced only short-lasting LTP in Sal003-treated hippocampal slices from WT mice (Fig. 5A) (Costa-Mattioli et al., 2007a). In further electrophysiological tests, L-LTP elicited in slices from ATF4 knockouts was resistant to Sal003, confirming that the increase in ATF4 levels mediates the inhibitory action of Sal003. Moreover, locally injected Sal003 increased eIF2α phosphorylation in the hippocampus of WT mice and impaired their learning and memory in the water maze (Fig. 5B) and during contextual fear conditioning (Costa-Mattioli et al., 2007a).

The ability to enhance memory formation by decreasing the levels of repressors of gene expression appears to be a widely conserved mechanism, from sea slugs to mammals. After injecting antibodies against the *Aplysia* homolog of ATF4, ApCREB2, into sensory neurons, a single pulse of 5-HT, which normally induces only short-term facilitation (lasting minutes), is sufficient to evoke a gene expression-dependent long-term facilitation that lasts beyond one day (Bartsch et al., 1995).

Our findings thus reveal a crucial step in mnemonic processing: the phosphorylation of a single site on eIF2α determines whether a STM process is transformed into a long-lasting one, through modulation of gene expression. A better understanding of the molecular basis of memory should lead to improved therapy of memory loss, whether associated with aging or, more devastatingly, with Alzheimer-type dementia.

Summary

Significant advances in studies of translational control of synaptic plasticity and memory formation have emerged in the last few years. GCN2-mediated phosphorylation of eIF2α and signaling downstream is an ancient signaling pathway, which is critical for the regulation of various biological processes. Recent well-integrated multidisciplinary approaches (molecular biology, genetics, electrophysiology, and behavior) have revealed the crucial role of eIF2α phosphorylation in synaptic plasticity and memory, thus providing new insights into the molecular mechanisms underlying synaptic plasticity and memory formation.

Acknowledgments

We thank Kresimir Krnjevic for comments on the manuscript. This work was supported by a Team Grant from the Canadian Institute of Health Research (CIHR) to M.C.-M and N.S. and a Howard Hughes Medical Institute (HHMI) grant to N.S.

References

Abel, T., Martin, K.C., Bartsch, D. and Kandel, E.R. (1998) Memory suppressor genes: inhibitory constraints on the storage of long-term memory. Science, 279: 338–341.

Agranoff, B.W. and Klinger, P.D. (1964) Puromycin effect on memory fixation in the goldfish. Science, 146: 952–953.

Anthony, T.G., McDaniel, B.J., Byerley, R.L., McGrath, B.C., Cavener, D.R., McNurlan, M.A. and Wek, R.C. (2004) Preservation of liver protein synthesis during dietary leucine deprivation occurs at the expense of skeletal muscle mass in mice deleted for eIF2 kinase GCN2. J. Biol. Chem., 279: 36553–36561.

Barco, A., Alarcon, J.M. and Kandel, E.R. (2002) Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. Cell, 108: 689–703.

Bartsch, D., Ghirardi, M., Skehel, P.A., Karl, K.A., Herder, S.P., Chen, M., Bailey, C.H. and Kandel, E.R. (1995) *Aplysia* CREB2 represses long-term facilitation: relief of repression converts transient facilitation into long-term functional and structural change. Cell, 83: 979–992.

Berlanga, J.J., Santoyo, J. and De Haro, C. (1999) Characterization of a mammalian homolog of the GCN2 eukaryotic initiation factor 2alpha kinase. Eur. J. Biochem., 265: 754–762.

Bliss, T.V. and Collingridge, G.L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. Nature, 361: 31–39.

Bourtchouladze, R., Abel, T., Berman, N., Gordon, R., Lapidus, K. and Kandel, E.R. (1998) Different training procedures recruit either one or two critical periods for contextual memory consolidation, each of which requires protein synthesis and PKA. Learn. Mem., 5: 365–374.

Boyce, M., Bryant, K.F., Jousse, C., Long, K., Harding, H.P., Scheuner, D., Kaufman, R.J., Ma, D., Coen, D.M., Ron, D. and Yuan, J. (2005) A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress. Science, 307: 935–939.

Carew, T.J., Hawkins, R.D. and Kandel, E.R. (1983) Differential classical conditioning of a defensive withdrawal reflex in *Aplysia californica*. Science, 219: 397–400.

Castellucci, V.F., Blumenfeld, H., Goelet, P. and Kandel, E.R. (1989) Inhibitor of protein synthesis blocks long-term behavioral sensitization in the isolated gill-withdrawal reflex of *Aplysia*. J. Neurobiol., 20: 1–9.

Chen, A., Muzzio, I.A., Malleret, G., Bartsch, D., Verbitsky, M., Pavlidis, P., Yonan, A.L., Vronskaya, S., Grody, M.B. Cepeda, I., et al. (2003) Inducible enhancement of memory storage and synaptic plasticity in transgenic mice expressing an inhibitor of ATF4 (CREB-2) and C/EBP proteins. Neuron, 39: 655–669.

Chen, J.J., Throop, M.S., Gehrke, L., Kuo, I., Pal, J.K., Brodsky, M. and London, I.M. (1991) Cloning of the cDNA of the heme-regulated eukaryotic initiation factor 2 alpha (eIF-2 alpha) kinase of rabbit reticulocytes: homology to yeast GCN2 protein kinase and human double-stranded-RNA-dependent eIF-2 alpha kinase. Proc. Natl. Acad. Sci. U.S.A., 88: 7729–7733.

Chong, K.L., Feng, L., Schappert, K., Meurs, E., Donahue, T.F., Friesen, J.D., Hovanessian, A.G. and Williams, B.R. (1992) Human p68 kinase exhibits growth suppression in yeast and homology to the translational regulator GCN2. EMBO J., 11: 1553–1562.

Costa-Mattioli, M., Gobert, D., Harding, H., Herdy, B., Azzi, M., Bruno, M., Bidinosti, M., Ben Mamou, C., Marcinkiewicz, E., Yoshida, M., et al. (2005) Translational control of hippocampal synaptic plasticity and memory by the eIF2alpha kinase GCN2. Nature, 436: 1166–1173.

Costa-Mattioli, M., Gobert, D., Stern, E., Gamache, K., Colina, R., Cuello, C., Sossin, W., Kaufman, R., Pelletier, J., Rosenblum, K., et al. (2007a) eIF2alpha phosphorylation bidirectionally regulates the switch from short- to long-term synaptic plasticity and memory. Cell, 129: 195–206.

Costa-Mattioli, M., Sonenberg, N. and Klann, E. (2007b) Translational control mechanism of synaptic plasticity and memory. In: Sweatt J.D. (Ed.), Learning and Memory: A Comprehensive Reference. Elsevier. (in press).

Crosby, J.S., Lee, K., London, I.M. and Chen, J.J. (1994) Erythroid expression of the heme-regulated eIF-2 alpha kinase. Mol. Cell. Biol., 14: 3906–3914.

Davis, H.P. and Squire, L.R. (1984) Protein synthesis and memory: a review. Psychol. Bull., 96: 518–559.

Deng, J., Harding, H.P., Raught, B., Gingras, A.C., Berlanga, J.J., Scheuner, D., Kaufman, R.J., Ron, D. and Sonenberg, N. (2002) Activation of GCN2 in UV-irradiated cells inhibits translation. Curr. Biol., 12: 1279–1286.

Dever, T.E. (2002) Gene-specific regulation by general translation factors. Cell, 108: 545–556.

Dever, T.E., Dar, A.C. and Sicheri, F. (2007) The eIF2alpha kinases. In: Mathews M.B., Sonenberg N. and Hershey J.W.B. (Eds.), Translational Control in Biology and Medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 319–345.

Doudna, J.A. and Sarnow, P. (2007) Translation initiation by viral internal ribosome entry sites. In: Mathews M.B., Sonenberg N. and Hershey J.W.B. (Eds.), Translational Control in Biology and Medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 129–154.

Duncan, R., Milburn, S.C. and Hershey, J.W. (1987) Regulated phosphorylation and low abundance of HeLa cell initiation factor eIF-4F suggest a role in translational control. Heat shock effects on eIF-4F. J. Biol. Chem., 262: 380–388.

Elroy-Stein, O. and Merrick, W.C. (2007) Translation initiation via cellular internal ribosome entry sites. In: Mathews M.B., Sonenberg N. and Hershey J.W.B. (Eds.), Translational Control in Biology and Medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 155–172.

Fanselow, M.S. and LeDoux, J.E. (1999) Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron, 23: 229–232.

Flexner, J.B., Flexner, L.B. and Stellar, E. (1963) Memory in mice as affected by intracerebral puromycin. Science, 141: 57–59.

Frey, U. and Morris, R.G. (1997) Synaptic tagging and long-term potentiation. Nature, 385: 533–536.

Frost, W.N., Castellucci, V.F., Hawkins, R.D. and Kandel, E.R. (1985) Monosynaptic connections made by the sensory neurons of the gill- and siphon-withdrawal reflex in *Aplysia* participate in the storage of long-term memory for sensitization. Proc. Natl. Acad. Sci. U.S.A., 82: 8266–8269.

Genoux, D., Haditsch, U., Knobloch, M., Michalon, A., Storm, D. and Mansuy, I.M. (2002) Protein phosphatase 1 is a molecular constraint on learning and memory. Nature, 418: 970–975.

Gietzen, D.W. (1993) Neuronal mechanism in the responses to amino acid deficiency. J. Nutr., 123: 610–625.

Gietzen, D.W., Ross, C.M., Hao, S. and Sharp, J.W. (2004) Phosphorylation of eIF2alpha is involved in the signaling of indispensable amino acid deficiency in the anterior periform cortex of the brain rats. J. Nutr., 134: 717–723.

Haberly, L.B. and Price, J.L. (1978) Association and commissural fiber systems of the olfactory cortex of the rat. J. Comp. Neurol., 178: 711–740.

Hao, S., Sharp, J.W., Ross-Inta, C.M., McDaniel, B.J., Anthony, T.G., Wek, R.C., Cavener, D.R., McGrath, B.C., Rudell, J.B., Koehnie, T.J. and Gietzen, D.W. (2005) Uncharged tRNA and sensing of amino acid deficiency in mammalian piriform cortex. Science, 307: 1176–1178.

Harding, H.P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M. and Ron, D. (2000) Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol. Cell, 6: 1099–1108.

Harding, H.P., Zhang, Y. and Ron, D. (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature, 397: 271–274.

Hinnebusch, A.G. (2006) eIF3: a versatile scaffold for translation initiation complexes. Trends Biochem. Sci., 31: 553–562.

Hinnebusch, A.G., Asano, K., Olsen, D.S., Phan, L., Nielsen, K.H. and Valasek, L. (2004) Study of translational control of eukaryotic gene expression using yeast. Ann. NY Acad. Sci., 1038: 60–74.

Hinnebusch, A.G., Dever, T.E. and Asano, K. (2007) Mechanism of translation initiation in yeast *Saccharomyces cerevisiae*. In: Mathews M.B., Sonenberg N. and Hershey J.W.B. (Eds.), Translational Control in Biology and Medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 225–268.

Jackson, R.J., Kaminski, A. and Poyry, T.A.A. (2007) Coupled termination-reinitiation events in mRNA translation. In: Mathews M.B., Sonenberg N. and Hershey J.W.B. (Eds.), Translational Control in Biology and Medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 197–224.

Kandel, E.R. (2001) The molecular biology of memory storage: a dialogue between genes and synapses. Science, 294: 1030–1038.

Kelleher, III, R.J., Govindarajan, A. and Tonegawa, S. (2004) Translational regulatory mechanisms in persistent forms of synaptic plasticity. Neuron, 44: 59–73.

Klann, E. and Dever, T.E. (2004) Biochemical mechanisms for translational regulation in synaptic plasticity. Nat. Rev. Neurosci., 5: 931–942.

Krug, M., Lossner, B. and Ott, T. (1984) Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. Brain. Res. Bull., 13: 39–42.

LeDoux, J.E. (2000) Emotion circuits in the brain. Annu. Rev. Neurosci., 23: 155–184.

Lu, P.D., Harding, H.P. and Ron, D. (2004) Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. J. Cell Biol., 167: 27–33.

Malenka, R.C. and Nicoll, R.A. (1999) Long-term potentization: a decade of progress? Science, 285: 1870–1874.

Malleret, G., Haditsch, U., Genoux, D., Jones, M.W., Bliss, T.V., Vanhoose, A.M., Weitlauf, C., Kandel, E.R., Winder, D.G. and Mansuy, I.M. (2001) Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. Cell, 104: 675–686.

Martin, K.C., Casadio, A., Zhu, H., Yaping, E., Rose, J.C., Chen, M., Bailey, C.H. and Kandel, E.R. (1997) Synapse-specific, long-term facilitation of *Aplysia* sensory to motor synapses: a function for local protein synthesis in memory storage. Cell, 91: 927–938.

Martin, K.C. and Kosik, K.S. (2002) Synaptic tagging: who's it? Nat. Rev. Neurosci., 3: 813–820.

Mathews, M.B., Sonenberg, N. and Hershey, J.W.B. (2007a) Origins and principles of translational control. In: Mathews M.B., Sonenberg N. and Hershey J.W.B. (Eds.), Translational Control in Biology and Medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1–40.

Mathews, M.B., Sonenberg, N. and Hershey, J.W.B. (2007b) Translational control in biology and medicine (2nd ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Maurin, A.-C., Jousse, C., Averous, J., Parry, L., Bruhat, A., Cherasse, Y., Zeng, H., Zhang, Y., Harding, H.P., Ron, D. and Fafournoux, P. (2005) The GCN2 kinase biases feeding behavior to maintain amino acid homeostasis in omnivores. Cell Metab., 1: 273–277.

Mellor, H., Flowers, K.M., Kimball, S.R. and Jefferson, L.S. (1994) Cloning and characterization of cDNA encoding rat hemin-sensitive initiation factor-2 alpha (eIF-2 alpha) kinase. Evidence for multitissue expression. J. Biol. Chem., 269: 10201–10204.

Meurs, E., Chong, K., Galabru, J., Thomas, N.S., Kerr, I.M., Williams, B.R. and Hovanessian, A.G. (1990) Molecular cloning and characterization of the human double-stranded RNA-activated protein kinase induced by interferon. Cell, 62: 379–390.

Montarolo, P.G., Goelet, P., Castellucci, V.F., Morgan, J., Kandel, E.R. and Schacher, S. (1986) A critical period for macromolecular synthesis in long-term heterosynaptic facilitation in *Aplysia*. Science, 234: 1249–1254.

Morris, R.G. and Frey, U. (1999) Tagging the Hebb synapse: reply. Trends Neurosci., 22: p. 256.

Morris, R.G., Garrud, P., Rawlins, J.N. and O'Keefe, J. (1982) Place navigation impaired in rats with hippocampal lesions. Nature, 297: 681–683.

Narasimhan, J., Staschke, K.A. and Wek, R.C. (2004) Dimerization is required for activation of eIF2 kinase Gcn2 in response to diverse environmental stress conditions. J. Biol. Chem., 279: 22820–22832.

Pestova, T.V., Lorsch, J.R. and Hellen, C.U.T. (2007) The mechanism of translation initiation in eukaryotes. In: Mathews M.B., Sonenberg N. and Hershey J.W.B. (Eds.), Translational Control in Biology and Medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 87–128.

Pinsker, H.M., Hening, W.A., Carew, T.J. and Kandel, E.R. (1973) Long-term sensitization of a defensive withdrawal reflex in *Aplysia*. Science, 182: 1039–1042.

Raught, B. and Gingras, A.C. (2007) Signaling to translation initiation. In: Mathews M.B., Sonenberg N. and Hershey J.W.B. (Eds.), Translational Control in Biology and Medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 369–400.

Ron, D. and Harding, H.P. (2007) eIF2alpha phosphorylation in cellular stress responses and disease. In: Mathews M.B., Sonenberg N. and Hershey J.W.B. (Eds.), Translational Control in Biology and Medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 345–368.

Santoyo, J., Alcalde, J., Mendez, R., Pulido, D. and de Haro, C. (1997) Cloning and characterization of a cDNA encoding a protein synthesis initiation factor-2alpha (eIF-2alpha) kinase from *Drosophila melanogaster*. Homology to yeast GCN2 protein kinase. J. Biol. Chem., 272: 12544–12550.

Schafe, G.E., Nadel, N.V., Sullivan, G.M., Harris, A. and LeDoux, J.E. (1999) Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. Learn. Mem., 6: 97–110.

Scoville, W.B. and Milner, B. (1957) Loss of recent memory after bilateral hippocampal lesions. J. Neurol. Neurosurg. Psychiatry., 20: 11–21.

Shi, Y., Vattem, K.M., Sood, R., An, J., Liang, J., Stramm, L. and Wek, R.C. (1998) Identification and characterization of pancreatic eukaryotic initiation factor 2 alpha-subunit kinase, PEK, involved in translational control. Mol. Cell. Biol., 18: 7499–7509.

Silva, A.J., Kogan, J.H., Frankland, P.W. and Kida, S. (1998) CREB and memory. Annu. Rev. Neurosci., 21: 127–148.

Sood, R., Porter, A.C., Olsen, D.A., Cavener, D.R. and Wek, R.C. (2000) A mammalian homologue of GCN2 protein kinase important for translational control by phosphorylation of eukaryotic initiation factor-2alpha. Genetics, 154: 787–801.

Squire, L.R. (1987) Short-term and long-term memory processes. Oxford University Press, New York.

Steward, O. and Schuman, E.M. (2001) Protein synthesis at synaptic sites on dendrites. Annu. Rev. Neurosci., 24: 299–325.

Steward, O. and Schuman, E.M. (2003) Compartmentalized synthesis and degradation of proteins in neurons. Neuron, 40: 347–359.

Sutton, M.A. and Schuman, E.M. (2006) Dendritic protein synthesis, synaptic plasticity, and memory. Cell, 127: 49–58.

Takei, N., Kawamura, M., Hara, K., Yonezawa, K. and Nawa, H. (2001) Brain-derived neurotrophic factor enhances neuronal translation by activating multiple initiation processes: comparison with the effects of insulin. J. Biol. Chem., 276: 42818–42825.

Vattem, K.M. and Wek, R.C. (2004) Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. Proc. Natl. Acad. Sci. U.S.A., 101: 11269–11274.

Yin, J.C., Del Vecchio, M., Zhou, H. and Tully, T. (1995) CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in *Drosophila*. Cell, 81: 107–115.