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Translational control by eIF2α kinases in long-lasting synaptic plasticity and long-term memory

Mimi A. Trinh [a], Eric Klann [b,*]

[a] Pharmaceutical Research Division, CNS Drug Discovery Unit, Takeda Pharmaceutical Company Limited, Fujisawa, Kanagawa 251-8555, Japan
[b] Center for Neural Science, New York University, New York, NY 10003, USA

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ABSTRACT

Although the requirement for new protein synthesis in synaptic plasticity and memory has been well established, recent genetic, molecular, electrophysiological, and pharmacological studies have broadened our understanding of the translational control mechanisms that are involved in these processes. One of the critical translational control points mediating general and gene-specific translation depends on the phosphorylation of eukaryotic initiation factor 2 alpha (eIF2α) by four regulatory kinases. Here, we review the literature highlighting the important role for proper translational control via regulation of eIF2 α phosphorylation by its kinases in long-lasting synaptic plasticity and long-term memory.

1. Introduction

One of the more remarkable features of the brain is the ability to acquire and store new information as lasting memory traces. This continuous capacity to learn and remember allows one to process changes in the environment, retain new information, and adapt to behavioral choices over time. A fundamental question remains that intrigues modern neuroscientists: how are memories formed and stored at the cellular and molecular level? Behavioral studies performed in mice treated with the protein synthesis inhibitor puromycin provided the first molecular clue that protein synthesis is required for long-term memory (LTM) formation, but not for task acquisition and short-term memory (STM) formation (Flexner, Flexner, & Stellar, 1963). Since then, a plethora of pharmacological and genetic studies have highlighted the critical role for *de novo* gene expression and protein synthesis in LTM formation (Kandel, 2001; McGaugh, 2000).

Neurons can alter their molecular and physiological characteristics in response to temporal- and activity-dependent changes in their environment. Synaptic plasticity refers to the ability of the brain to change the efficacy (strengthening or weakening) of synaptic connections between neurons and is hypothesized as the cellular basis for learning and memory (Bliss & Collingridge, 1993; Malenka & Nicoll, 1999). These persistent, activity-dependent, changes in synaptic strength are triggered by *de novo* protein synthesis (Klann & Sweatt, 2008). Evidence indicating a role for protein synthesis at local synaptic sites stem from observations that neuronal dendrites and their spines contain polyribosomes (Steward & Levy, 1982), translation factors (Tang & Schuman, 2002), and mRNA (Crino & Eberwine, 1996) that can be translated into proteins to support synaptic activity. Consistent with this notion, local protein synthesis was shown to be necessary for long-lasting increases in synaptic strength induced by brain-derived neurotrophic factor (BDNF; Kang & Schuman, 1996). Similarly, rapid, local protein synthesis also was required for long-lasting decreases in synaptic strength induced by activation of group I metabotropic glutamate receptors (mGluR; Huber, Kayser, & Bear, 2000). Together, these findings indicate that protein synthesis can be triggered locally at activated synapses and is required for persistent, activity-dependent forms of synaptic plasticity, which in turn is thought to be essential for memory formation.

Although the initial report from Flexner et al. (1963) and other early studies identified new protein synthesis as a molecular requirement for memory formation, they offered little in the way of molecular translational control mechanisms because they relied mostly on the administration of general translation inhibitors into animals. In the last 10 years, however, a vast amount of genetic, biochemical, pharmacological, and physiological studies have increased our knowledge of the precise translational control mechanisms underlying long-lasting synaptic plasticity, memory formation, and cognitive function (Costa-Mattioli, Sossin, Klann, & Sonenberg, 2009; Kelleher, Govindarajan, & Tonegawa, 2004;

* Corresponding author. Address: Center for Neural Science, New York University, 4 Washington Place, Room 809, New York, NY 10003, USA.
E-mail address: eklann@cns.nyu.edu (E. Klann).

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Richter & Klann, 2009). In this review, we specifically discuss the functional role of eIF2α kinases and their regulation of activity-dependent synaptic plasticity and cognitive function, including learning and memory.

2. Translational control by eIF2α phosphorylation

Translational control can be defined as a change in either the efficiency or rate of mRNA translation. The process of mRNA translation can be divided into three main steps: initiation, elongation, and termination. Although regulation can occur at each step, translational control primarily occurs at the rate-limiting initiation step when the small 40S ribosomal subunit is recruited to the mRNA and positioned at the initiation codon (Jackson, Hellen, & Pestova, 2010). Translation initiation itself can be further divided into three key steps. First, the initiator methionyl transfer RNA (Met-tRNA$_i^{Met}$) binds to the small 40S ribosomal subunit, forming the 43S preinitiation complex. This is followed by the binding of the 43S complex to the mRNA so that it can find the initiation codon, thereby forming the 48S complex. Finally, the large ribosomal subunit joins the 48S complex to generate an 80S translational-competent ribosome, which can subsequently proceed with elongation (Jackson et al., 2010; Pestova, Lorsh, & Hellen, 2007).

One highly conserved mechanism of translational control in eukaryotic cells involves phosphorylation of eukaryotic initiation factor 2 (eIF2). In this early step in translation initiation, eIF2, a heterotrimer consisting of α, β, and γ subunits, binds Met-tRNA$_i^{Met}$ and GTP to form the stable 43S preinitiation complex (eIF2-GTP-Met-tRNA$_i^{Met}$). Exchange of GDP for GTP is promoted by eIF2B, a guanine-nucleotide exchange factor that is required to regenerate the active GTP-bound eIF2 that is required for new rounds of translation. The guanine-nucleotide exchange on eIF2 serves as a critical translational control point and is regulated via phosphorylation. Specifically, the phosphorylation of eIF2 on its α subunit at serine 51 (Ser51) converts eIF2 to a competitive inhibitor of eIF2B, which blocks the GDP/GTP-exchange and causes a decrease in general translation initiation (Pestova et al., 2007; Sonenberg & Dever, 2003).

Although eIF2α phosphorylation inhibits general translation, it also selectively increases the translation of a subset of mRNAs that contain upstream open reading frames (uORFs) in their 5′ untranslated region (UTR). uORFs are present in nearly half of rodent and human transcripts (Iacono, Mignone, & Pesole, 2005; Matsui, Yachie, Okada, Saito, & Tomita, 2007; Mignone, Gissi, Liuni, & Pesole, 2002), but despite their prevalence, they are less frequent than expected by chance (Iacono et al., 2005), and also are highly conserved (Neafsey & Galagan, 2007).

Probably the best characterized example of gene-specific translational control via eIF2α phosphorylation is that of the yeast transcriptional activator GCN4 (Hinnebusch & Natarajan, 2002). When general translation was inhibited by eIF2α phosphorylation, GCN4 translation, as well as the translation of the transcriptional modulator ATF4 (activating transcription factor 4; also termed CREB2) was enhanced (Harding et al., 2000; Vattem & Wek, 2004). Additional uORF-containing mRNAs have been shown to be translated under conditions resulting in enhanced eIF2α phosphorylation, including the CAAT/enhancer binding proteins C/EBPα and β (Calkhoven, Muller, & Leutz, 2000) and the β-site β-amyloid precursor protein (APP)-cleaving enzyme BACE1 (De Pietri Tonelli et al., 2004; Lammich, Schobel, Zimmer, Lichtenthaler, & Haass, 2004). Notably, in multiple species ATF4 and its homologs act as repressors of cAMP-responsive element binding protein (CREB)-mediated gene expression, which is known to be required for long-lasting changes in synaptic plasticity and LTM (Abel, Martin, Bartsch, & Kandel, 1998; Bartsch et al., 1995; Chen et al., 2003). Thus, eIF2α phosphorylation controls both general and gene-specific translation that regulates CREB-mediated transcription, two distinct processes that are required for long-lasting synaptic plasticity and LTM formation.

3. eIF2α kinases

eIF2α phosphorylation is regulated by four serine/threonine (Ser/Thr) protein kinases, each of which phosphorylate eIF2α on Ser51. The four eIF2α kinases are heme-regulated inhibitor (HRI), the double-stranded (ds) RNA activated protein kinase (PKR), the general control non-derepressible-2 (GCN2), and the PKR-like endoplasmic reticulum (ER) resident protein kinase (PERK). These four eIF2α kinases share a conserved kinase domain, but respond differentially to various cellular stressors due to divergent regulatory domains (Dever, Dar, & Sicheri, 2007). For example, HRI is activated by conditions of heme deficiency (Mellor, Flowers, Kimball, & Jefferson, 1994), PKR is activated by double-stranded RNA (dsRNA; Meurs et al., 1990), GCN2 is activated by amino acid deprivation as well as UV irradiation (Deng et al., 2002; Sood, Porter, Olsen, Cavener, & Wek, 2000), and PERK is activated by an accumulation of misfolded proteins in the ER (Harding, Zhang, & Ron, 1999; Shi et al., 1998). Thus, depending on the particular cellular stimuli, specific eIF2α kinases become active and phosphorylate eIF2α to control both general and gene-specific translation (Fig. 1). There is very little information concerning whether these kinases are activated under normal physiological conditions, especially in neurons.

All eIF2α kinases are abundantly expressed in the mammalian brain (Berlanga, Santoyo, & De Haro, 1999; Harding et al., 1999; Meurs et al., 1990; Shi et al., 1998; Sood et al., 2000; Trinh et al., 2012; Zhu et al., 2011), with the exception of HRI whose expression is relatively low (Crosby, Lee, London, & Chen, 1994; Mellor et al., 1994). We will describe the most salient aspects of GCN2, PKR, and PERK because they are known to play important roles in protein-synthesis dependent synaptic plasticity and cognitive function, including learning and memory.

GCN2 is present in all eukaryotes (Dever et al., 2007; Hinnebusch et al., 2004; Sood et al., 2000) and is activated in response to amino acid starvation via the accumulation of uncharged tRNAs. GCN2's structure is complex and contains five domains: (1) an N-terminal domain that binds to GCN1 and is required for activation, (2) a pseudokinase domain, (3) an eIF2α kinase domain, (4) a regulatory domain resembling histidyl-tRNA synthetase (HisRS), containing a conserved sequence (motif 2), which is thought to bind all deacylated tRNAs with similar affinity, and (5) a carboxy-terminal domain that dimerizes, enhances tRNA binding, and mediates ribosomal binding (Dever et al., 2007). In contrast to the monomers PKR and PERK that require dimerization for activation, GCN2 exists constitutively as a dimer where the HisRS domain interacts with both the kinase domain and the carboxy-terminal domain to maintain it in inactive state. In response to amino acid starvation, uncharged tRNAs accumulate and bind to the HisRS domain, which results in the release of these inhibitory interactions and the subsequent activation of GCN2 (Dever et al., 2007). GCN2 also is activated by UV irradiation, high salinity, rapamycin, and glucose limitation (Deng et al., 2002; Hinnebusch, 2005). Interestingly, these stress stimuli could not activate a GCN2 mutant lacking a functional HisRS domain (Hinnebusch, 2005; Wek, Zhu, & Wek, 1995). All together, these findings indicate that uncharged tRNA is the main activator of GCN2.

PKR is expressed widely in vertebrates and activated in response to dsRNA produced during viral infection (Dever et al., 2007). Compared to GCN2 and PERK, the structure of PKR is relatively simple with an N-terminal dsRNA-binding domain (dsRBD), which consists of two dsRNA binding motifs (dsRBMs), and a car-

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