The Maintenance of LTP by PKMζ

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Abstract

This paper reviews evidence for the role of PKMζ in maintaining long-term potentiation (LTP) and discusses whether or not the very long term memory (VLTM, > 1 month) is stored in PKMζ. The LTP induction may cause protein kinase A (PKA) to translocate from spines to the nucleus, triggering expression of the brain-derived neurotrophic factor (BDNF), which subsequently induces production of plasticity-related proteins, including PKMζ - a persistently active atypical protein kinase C (aPKC). Once synthesized near the synapse, the active PKMζ may up-regulate the GluA2-containing AMPARs to maintain LTP. On the other hand, the translation of PKMζ is negatively regulated by the α-subunit of eukaryotic initiation factor 2 (eIF2α), which in turn is controlled by protein kinase-like ER kinase (PERK). PKMζ was thought to store VLTM based on the finding that its inhibitor, zeta inhibitory peptide (ZIP), abolishes the memory of conditioned taste aversion even 3 mo after encoding (Shema et al., 2009). Recent studies suggest that the ZIP dosage used by Shema et al. could also inhibit PKCλ, thereby disrupting microtubule transport of PSD-95. According to the Microtubule Track (MTT) hypothesis for VLTM, disruption of PSD-95 trafficking along specific MTTs should abolish the memory.

Introduction

Long-term potentiation (LTP) contains two stages: induction and maintenance. The mechanism of LTP induction has been largely unveiled (Paper 17), but how the potentiation is maintained remains controversial (Lisman, 2017). Two competing hypotheses have been proposed. One of

them centers on atypical protein kinase C (aPKC) such as PKMζ and PKCι/λ (Sacktor, 2012), and the other focuses on Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) (Sanhueza and Lisman, 2013). This paper will show that the currently available evidence supports the aPKC hypothesis.

The CaMKII Hypothesis

Before activation, CaMKII is mostly associated with F-actin (see this figure) which limits the entry of CaMKII into the postsynaptic density (PSD) - a structure just beneath the postsynaptic membrane. Upon Ca^{2+}/calmodulin binding that induces autophosphorylation at T286, the association between CaMKII and F-actin is interrupted, allowing CaMKII to enter PSD and bind to the GluN2B (also known as NR2B) subunit of NMDA receptor (NMDAR). Their interaction locks CaMKII in a persistently active conformation even in the absence of Ca^{2+}/calmodulin binding or autophosphorylation (Bayer et al., 2001). This led to the assumption that synaptic strength might be stored stably in the CaMKII/NMDAR complex (Sanhueza and Lisman, 2013). The hypothesis, however, faces several challenges:

1. Inhibition of postsynaptic CaMKII blocks induction but not maintenance of LTP (Malinow et al., 1989).
2. Induction of LTP in single spines triggered transient (approximately 1 min), rather than sustained, CaMKII activation (Lee et al., 2009).
3. The CaMKII–NMDAR binding can be disrupted by the endogenous inhibitor CaMKIIN (Gouet et al., 2012), which is up-regulated within 30 minutes after learning (Lepicard et al., 2006).
4. The CaMKII phosphorylated at T305 has been demonstrated to dissociate from PSD, thus blocking LTP and learning (Elgersma et al., 2002). Six hours after classical conditioning, the level of T305 phosphorylation is significantly elevated, while T286 phosphorylation decreases to the basal level (Naskar et al., 2014).
5. Prolonged ligand binding on the NMDAR may cause protein phosphatase 1 to dephosphorylate T286, consequently leading to synaptic **depression** (Dore et al., 2016).

Evidence for a Role of PKMζ

Over the past two decades, a large number of studies have provided compelling evidence that PKMζ plays a key role in LTP maintenance. Major findings include:

1. Unlike CaMKII, whose association with NMDARs is interrupted shortly after LTP induction, the PKMζ protein level increases persistently for up to a month (Sacktor et al., 1993; Hsieh et al., 2017).
2. Injection of the PKMζ inhibitor, zeta inhibitory peptide (ZIP), into rat cortex abolishes long-term memory (Shema et al., 2007).
3. Knockdown of PKMζ in the hippocampus impairs LTP maintenance and disrupts previously established long term memory (Wang et al., 2016).
4. PKMζ-specific antisense oligonucleotide that prevents its up-regulation impaired the LTP maintenance and memory, suggesting the necessity of the up-regulation (Yu et al., 2017).
5. Overexpression of PKMζ enhances long-term potentiation and long-term memory (Shema et al., 2011; Xue et al., 2015; Schuette et al., 2016).

In 2013, while evidence for the crucial role of PKMζ in LTP maintenance was accumulating, two independent groups reported that knockout of the gene encoding PKMζ did not have any significant impact on learning and memory (Lee et al., 2013; Volk et al., 2013). This appeared to be a serious blow to the PKMζ hypothesis. Fortunately, it turns out that the other atypical protein kinase C, PKCι/λ, may compensate for the function of PKMζ (Tsokas et al., 2016). However, under physiological conditions, PKMζ and PKCι/λ play distinct roles: PKMζ is responsible for LTP maintenance while PKCι/λ contributes to early LTP (Wang et al., 2016). PKCι/λ is employed to maintain LTP only if PKMζ malfunctions (Sacktor and Hell , 2017).

The above situation is similar to CRMP2, which could play a key role in memory extinction and consolidation (to be discussed in later papers). The antibody against CRMP2 has been demonstrated to induce amnesia (Mileusnic and Rose, 2011), but deletion of the CRMP2 gene causes only mild memory deficits (Nakamura et al., 2016; Zhang et al., 2016). The CRMP family includes five members (Schmidt and Strittmatter, 2007). Other members are likely to compensate for the loss of CRMP2, whereas the anti-CRMP2 antibody may act on most CRMP members.

Downstream Effects on Synaptic AMPARs

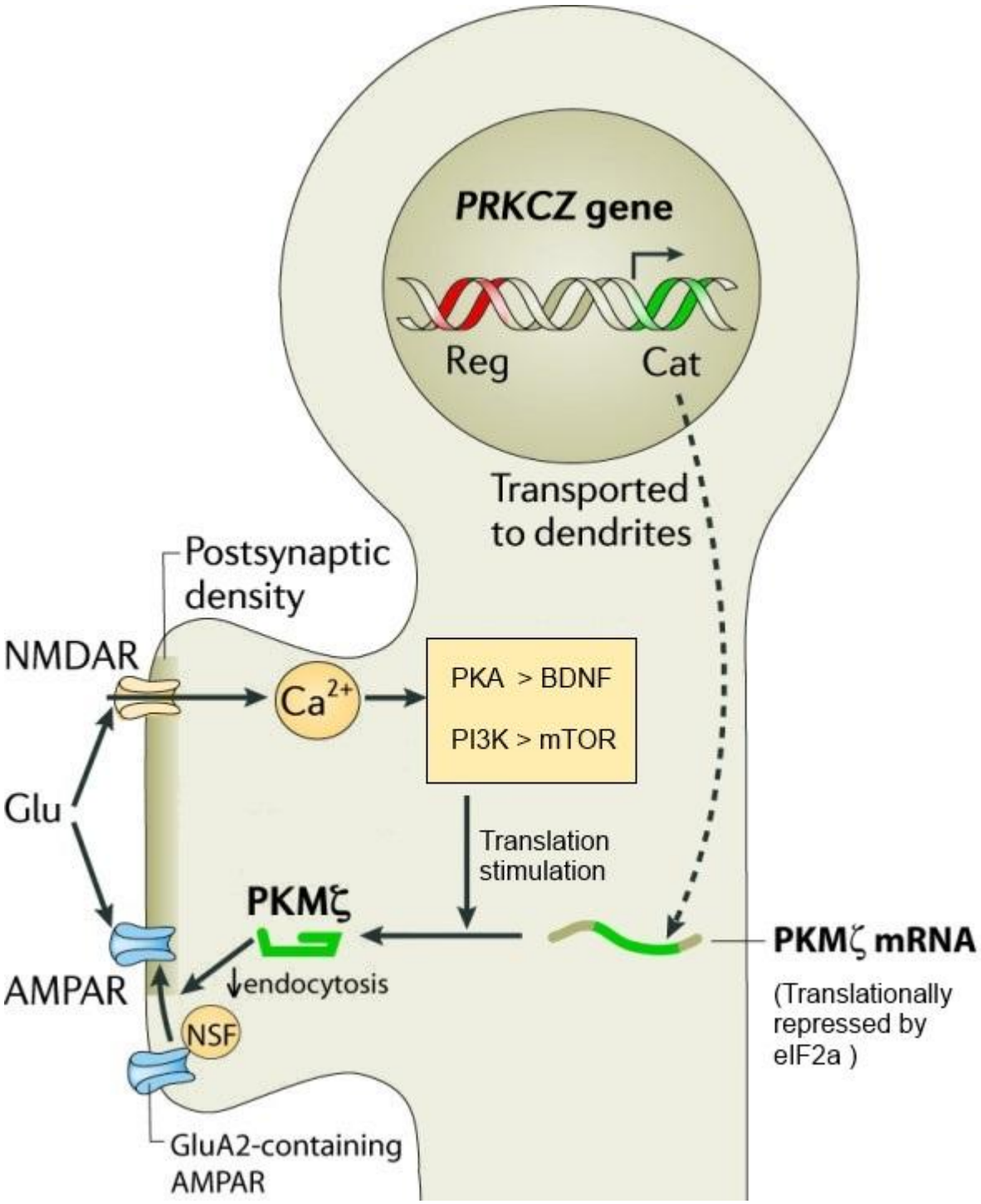

Figure 1. The mechanism underlying PKMζ-mediated LTP maintenance. Before LTP induction, the PKMζ mRNA is translationally repressed by eIF2α. Upon strong synaptic stimulation, the Ca^{2+} influx through NMDARs may activate protein kinase A (PKA) to trigger the production of BDNF, which subsequently stimulates the translation of PKMζ mRNA

through the PI3K-mTOR pathway. The newly synthesized PKMζ is persistently active. It may maintain elevated synaptic AMPAR level by decreasing receptor endocytosis through an NSF-dependent pathway and immobilization of synaptic AMPARs. [Adapted from: Sacktor, 2012]

How can PKMζ maintain LTP? Fundamentally, LTP is manifested in the increase of synaptic AMPARs. PKMζ is a persistently active protein kinase. Once synthesized near the synapse, the active PKMζ may up-regulate GluA2-containing AMPARs by decreasing receptor endocytosis mediated by N-ethylmaleimide-sensitive factor (NSF) (Figure 1). In addition, PKMζ has the capacity to impede lateral movement of GluA2-containing AMPARs at the synapse, thereby increasing synaptic AMPARs (Yu et al., 2017).

Upstream Regulation by mTOR and eIF2α

How is PKMζ regulated during LTP? Both phosphoinositide 3-kinase (PI3K) and mechanistic target of rapamycin (mTOR) are implicated in the synthesis of PKMζ from its mRNA (Kelly et al., 2007). The PI3K-mTOR axis is a canonical pathway for protein synthesis from mRNA. Upon activation, mTOR may phosphorylate two major targets, p70 ribosome S6 kinase1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4EBP1), resulting in the initiation of protein synthesis (Paper 3). As mentioned in Paper 17, the LTP induction may cause PKA to translocate from spines to the soma. Inside the nucleus, PKA is known to trigger gene expression by phosphorylating cAMP response element-binding protein (CREB) (Delghandi et al., 2005). The most important PKA target is brain-derived neurotrophic factor (BDNF). Once expressed, BDNF may further stimulate the BDNF-TrkB signaling pathways to produce plasticity-related proteins, including PKMζ (Adasme et al., 2011).

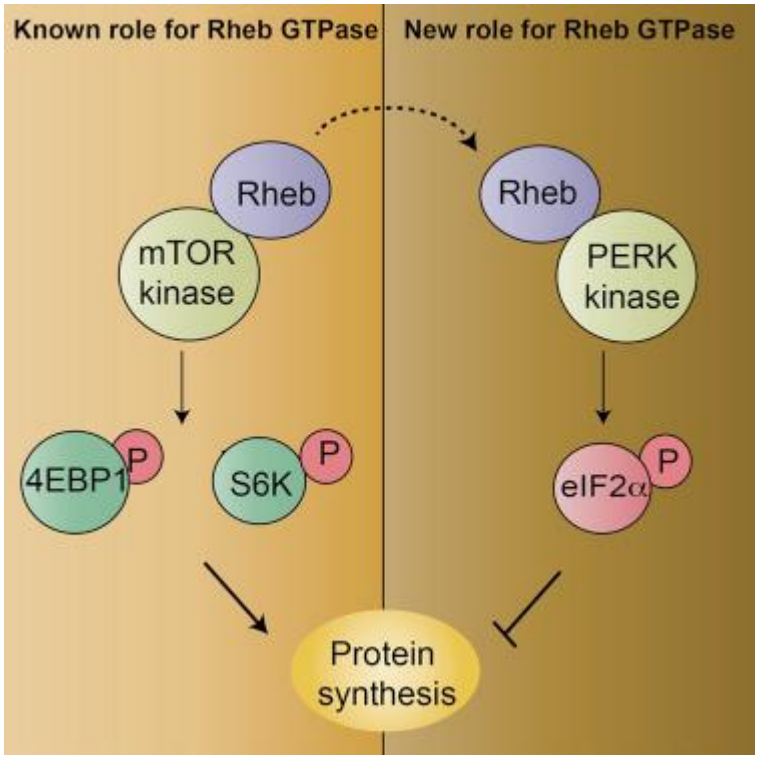

Figure 2. Translational control by Rheb. Binding of Rheb to mTOR promotes, whereas to PERK inhibits, protein synthesis [Source: Tyagi et al., 2015]

While the mTOR pathway up-regulates PKMζ, the α-subunit of eukaryotic initiation factor 2 (eIF2α) may down-regulate PKMζ by repressing the translation of its mRNA (Chesnokova et al., 2017). The phosphorylation of eIF2α has been shown to inhibit general translation, but selectively stimulates translation of ATF4, a repressor of CREB-mediated late-LTP (Costa-Mattioli et al., 2005; Costa-Mattioli et al., 2007). Four kinases are known to be capable of phosphorylating eIF2α. One of them is protein kinase-like ER kinase (PERK). In an animal model of Alzheimer's disease, PKMζ was down-regulated, but conditional deletion of PERK restored PKMζ expression back to the normal level (Ma et al., 2013), suggesting that PERK may down-regulate PKMζ by phosphorylating eIF2α. This notion is consistent with a recent finding that Rheb, a crucial protein in the PI3K-mTOR axis, controls the switch between translational stimulation and repression (Figure 2). Binding of Rheb to mTOR promotes, whereas to PERK inhibits, protein synthesis (Tyagi et al., 2015).

The Engram of Very Long-Term Memory

In the literature, the term "long-term memory" usually refers to the memory that can last longer than one day. However, there is a clear difference between the labile memories that are being consolidated and the stable memories after consolidation. To avoid ambiguity, the former will be called "transitional memory" and the latter "very long-term memory" (VLTM). The transitional memory may last up to a month since the consolidation process takes about a month (Bontempi et al., 1999).

PKMζ was thought to store VLTM as ZIP abolishes the memory of conditioned taste aversion even 3 mo after encoding, when consolidation is presumably complete (Shema et al., 2009). A recent study, however, demonstrated that ZIP could impair the learning-induced memory if applied a few days after learning, but had no effect if applied two weeks or a month later (Hales et al., 2015; Hales et al., 2016). The discrepancy may arise from the dosage of ZIP. It has been shown that at certain concentration, ZIP inhibits only PKMζ, not PKCλ. As the concentration increases, ZIP begins to display additional inhibitory effect on PKCλ (Ren et al., 2013).

According to the Microtubule Track (MTT) hypothesis, VLTM is encoded in specific microtubule tracks directing PSD-95 trafficking from the soma toward potentiated spines (Lee, 2009; Lee, 2013a; Lee, 2013b). The microtubule transport of PSD-95 requires phosphorylation of the palmitoylation enzyme (e.g., ZDHHC8) by conventional or atypical PKC. While both chelerythrine (an inhibitor of conventional PKC) and ZIP suppressed the postsynaptic localization of PSD-95, RNA interference for PKMζ did not have a significant effect, suggesting that the ZIP peptide may block an atypical PKC other than PKMζ (Yoshii et al., 2014). Therefore, in the study of Shema et al., ZIP could inhibit both PKMζ and PKCλ. The inhibition of PKCλ may reduce the activity of ZDHHC8, resulting in suppression of PSD-95 trafficking along microtubule tracks. This consequence, according to the MTT hypothesis, should abolish encoded VLTM. By contrast, in the study of Hales et al., ZIP could inhibit only PKMζ whose primary function is to maintain an elevated level of synaptic AMPARs while the memory is being consolidated. The next paper will explain why elevated synaptic AMPARs are necessary during memory consolidation.

An Interesting Analogy

Below is the comment of an anonymous reviewer on the manuscript of Tsokas et al. (2016) before it was published in the journal *Elife*.

> I fear that the senior author's view on this is *not* shared by others as a third possibility is not considered – namely that a molecule implicated in maintenance, while to be sure not contributing to initial memory formation, might not be sustained throughout the lifetime of a memory. To the contrary, it may trigger structural changes mediated by other molecules, and then depart the scene and no longer be involved. .... An analogy might be helpful here. Consider a space rocket already in space that is circling the earth and is to be sent to the moon. For a brief period, the thrusters are activated and the rocket escapes earth's gravity and speeds up. It is on its way to the moon. The engines are stopped and the rocket keeps going. Should we look for the 'molecules' that sustain its motion towards the moon, akin to maintaining a memory as in Sacktor's argument? Or do we recognise that Newton's Laws of Motion distinguish acceleration and velocity and recognise that nothing is needed to sustain velocity in space? The molecules that make memory retention possible could, like the engines on a rocket, be activated only briefly.

Could the "structural changes" be the construction of microtubule tracks?

Testable Prediction

PKCλ, but not PKMζ, may phosphorylate ZDHHC8 to promote PSD-95 trafficking along specific microtubule tracks from the soma toward potentiated spines.