Reciprocal activation within a kinase effector complex: A mechanism for the persistence of molecular memory

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ABSTRACT

Synaptic connections in neuronal circuits change in response to neuronal activity patterns. This can induce a persistent change in the efficacy of synaptic transmission, a phenomenon known as synaptic plasticity. One form of plasticity, long-term potentiation (LTP) has been extensively studied as the cellular basis of memory. In LTP, the potentiated synaptic transmission persists along with structural changes in the synapses. Many studies have sought to identify the “memory molecule” or the “molecular engram”. Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is probably the most well-studied candidate for the memory molecule. However, consensus has not yet been reached on a very basic aspect: how CaMKII is regulated during LTP. Here, I propose a new model of CaMKII regulation: reciprocal activation within a kinase effector complex (RAKEC) that is made between CaMKII and its effector protein, which is mediated by a persistent interaction between CaMKII and a pseudosubstrate sequence on T-lymphoma invasion and metastasis protein 1 (Tiam1), resulting in reciprocal activation of these two molecules. Through the RAKEC mechanism, CaMKII can maintain memory as biochemical activity in a synapse-specific manner. In this review, the detailed mechanism of the RAKEC and its expansion for the maintenance of LTP is described.

1. Introduction

Connectivity in brain neuronal circuits changes in response to activity input in a process, which is believed to be the cellular counterpart of memory storage in the brain tissue. The functional changes in the synaptic connectivity are accompanied by structural changes in stimulated synapses in both in vivo and ex vivo model systems (Bosch and Hayashi, 2012; Okamoto et al., 2004). As some memories can last for an individual’s lifetime, it is important to understand how memory is stored in the brain tissue.

Two of the most studied synaptic plasticity mechanisms are long-term potentiation (LTP) and long-term depression (LTD). LTP is a form of plasticity in which synapses that receive a high frequency of synaptic activity respond by increasing the strength of the synapse long-term. Conversely, LTD is characterized by a long-lasting decrease in synaptic strength. These forms of plasticity are not only found in in vivo settings but also in vitro (Whitlock et al., 2006; Gruart et al., 2006; Gruart et al., 2015). During LTP induction, calcium influx via N-methyl-D-aspartate (NMDA)-type glutamate receptors (NMDARs) leads to activation and translocation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) to synapses (Lisman et al., 2012; for review). CaMKII is an oligomeric multifunctional protein kinase family protein that is encoded on four genes: CaMKIIα, CaMKIIβ, CaMKIIγ, and CaMKIIδ. The predominant isoform in the brain tissue, CaMKIIα, is the most abundant protein in the postsynaptic densities of the excitatory synapses in the hippocampus (Lowenthal et al., 2015).

CaMKII is activated by binding of Ca^{2+}/calmodulin, resulting in its autophosphorylation on T286, making it a constitutively active kinase (Saitoh and Schwartz, 1985; Miller et al., 1988; Kennedy, 1989). In other words, Ca^{2+} rises via NMDARs, and this transient rise (Carter and Sabatini, 2004; Lee et al., 2009; Noguchi et al., 2005) is converted into persistent kinase activity of CaMKII through autophosphorylation. Due to its unique regulatory properties and its abundance in the brain tissue, CaMKII has become an attractive candidate for a “memory molecule” (Lisman and Goldring, 1988). Although the importance of CaMKII in synaptic plasticity and learning has been supported by various lines of investigation, including genetically modified animals, the regulation of CaMKII or its target for LTP have not yet been fully demonstrated. In this review, the molecular mechanisms underlying long-lasting functional and morphological changes in synapses that maintain LTP in excitatory neurons are reviewed.

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2. Structure of CaMKII

CaMKII consists of three different domains: the catalytic, regulatory, and association domains. The regulatory domain includes partly overlapping autoinhibitory and calmodulin binding sequences. The unique property of the catalytic domain of CaMKII is that it contains a canonical substrate interacting domain (S-site) and an autoregulatory segment (T-site); the T-site interacts with the T286 segment to act as a pseudosubstrate to maintain the inactivity of the kinase (Bayer and Schulman, 2019; Hell, 2014; Wayman et al., 2008) (Fig. 1). Two different binding mechanisms make CaMKII oligomeric: catalytic domains dimerize through substrate interacting domain (S-site) and an autoregulatory segment (T-site); the T-site interacts with the T286 segment to act as a pseudosubstrate to maintain the inactivity of the kinase (Bayer and Schulman, 2019; Hell, 2014; Wayman et al., 2008) (Fig. 1). Two different binding mechanisms make CaMKII oligomeric: catalytic domains dimerize and association domains assemble subunits; thus, CaMKII consists of dimers of hexamers or asymmetric heptamers, in total 12- to 14-mers as a holoenzyme (Rosenberg et al., 2005; Bhattacharyya et al., 2016); but see (Myers et al., 2017).

3. Is CaMKII activity required for maintenance?

CaMKII is activated by binding of Ca\(^{2+}\)/calmodulin and subsequent autophosphorylation on T286 via an adjacent kinase subunit within the same oligomer (Mukherji and Soderling, 1994), leading to Ca\(^{2+}\)/calmodulin-independent activity (autonomous activity) even after the Ca\(^{2+}\) concentration subsides. Because the T286 phosphorylation of CaMKII lasts longer than Ca\(^{2+}\) elevation during LTP (Barria et al., 1997; Ouyang et al., 1997, 1999), the generation of stimulation-independent activity fits the ideal model of “molecular memory.” Genetically modified animal studies of CaMKIIa, which include knockout (Silva et al., 1992) and knock-in of the kinase-dead point mutation (Yamagata et al., 2009) and knock-in of the autophosphorylation site, T286A, in mice (Giese et al., 1998), have reported impaired or reduced LTP and learning, supporting the conclusion of CaMKII as a memory molecule.

However, this view has been challenged by several findings. Application of CaMKII inhibitor was found to block LTP induction prior to stimulation, while application of the inhibitor after LTP was established could not reverse LTP (Chen et al., 2001; Malinow et al., 1989; Otma-khov et al., 1997). The newer peptide inhibitor, tatCN21, also had no effect on LTP maintenance at the concentration that specifically inhibits the kinase activity of CaMKII but not other protein interactions (Buard et al., 2010). Two-photon fluorescence-lifetime imaging microscopy (2pFLIM) with a fluorescence resonance energy transfer (FRET)-based CaMKIIa sensor revealed that CaMKIIa activation persisted for only 1 min during LTP induction by uncaging of caged-glutamate stimulation (Lee et al., 2009; Takao et al., 2005). Recently, optogenetic inhibition of CaMKII activity via photo-activatable CaMKII inhibitory peptide (PA-AIP2) has demonstrated that the requirement of CaMKIIa activation is transient during LTP and learning (Murakoshi et al., 2017). While simultaneous photoactivation of PA-AIP2 and LTP stimulation by glutamate uncaging impaired structural LTP induction, photodeactivation at a 30-sec to 1-min delay to uncaging failed to inhibit LTP, indicating that CaMKII activation is required during induction but not during maintenance, a result that was consistent with the results of the 2pFLIM imaging of CaMKII activity study (Murakoshi et al., 2017). Furthermore, in a study that used T286A knock-in CaMKIIa mutant mice, it was found that structural as well as functional LTP could be still induced by high-frequency stimulation in mutant neurons, indicating that autonomous activity via T286 phosphorylation of CaMKIIa is dispensable for LTP (Chang et al., 2017) and suggesting that CaMKII activation does not have to be persistent.

The involvement of protein kinase during LTP has been suggested in studies that used small molecule inhibitors. Pharmacological inhibition of CaMKII activity has been reported to reduce synaptic transmission by staurosporine and H-7 (Malinow et al., 1988); however, these compounds lack specificity to CaMKII. For example, they also inhibit protein kinase C (PKC) (Bain et al., 2007; Barcomb et al., 2013). Fukunaga et al. (1993) found elevated CaMKII enzymatic activity 60 min after LTP stimulation. Consistent with this finding, phosphorylated T286 of CaMKIIa was found to be increased in LTP-stimulated slices by western blotting and immunocytochemistry (Barria et al., 1997; Ouyang et al., 1997, 1999). However, the autonomous activity of CaMKII after LTP induction remains controversial (Fukunaga 1993; Lengyl et al., 2004). Fukunaga et al. (1993) showed that elevated CaMKII activity lasts in LTP-induced hippocampal slices, whereas Lengyl et al. (2004) did not detect autonomous CaMKIIa kinase activity in LTP-induced slices. A crucial step in kinase assays from brain slices is solubilizing the CaMKII in postsynaptic densities (PSDs) to test enzymatic activity. Given that PSD proteins, including CaMKII, are insoluble in TritonX-100, samples from kinase assays that use TritonX-100 might not contain CaMKII from PSD, and activated CaMKII could be diluted by inactive CaMKII from the cytosol (Fukunaga 1993; Lengyl et al., 2004). Additionally, upregulation of the CaMKIIa protein after LTP could further dilute activated CaMKIIa (Ouyang et al., 1999). Likewise, CaMKII activities measured using the FRET-based CaMKII biosensor (Takao et al., 2005; Lee et al., 2009) could be diluted by inactive CaMKII from the cytosol. Thus, it is important to examine CaMKIIa kinase activities from stimulated synapses by means of an optical method such as a subsynaptic domain-targeted probe combined with super-resolution microscopy.

A recent report from Rossetti et al. (2017) demonstrated that transiently expressed kinase-dead CaMKIIa (K42M) erased the memory of place avoidance tasks 16 days after training in vivo, indicating that CaMKIIa activity is required to maintain spatial memory. Transiently expressed kinase-dead CaMKIIa using herpes simplex virus did not dismantle the structure of the PSD, because animals could relearn the place avoidance task (Rossetti et al., 2017). However, the specificity of memory erasure by CaMKIIa K42M remains unclear. As the study did not monitor the expression of the mutant CaMKIIa during the experiment, it remains unknown whether the over-expressed CaMKIIa mutant was replaced into CaMKIIa holoenzymes in the potentiated synapses, and the expression of the mutant at the time of testing is also unclear.

In this way, whether persistent activity of CaMKII stores a memory in vitro or in vivo remains enigmatic. The critical targets of CaMKIIa required...
for long-term memory maintenance are yet to be identified. Therefore, the exact role of CaMKII in synaptic plasticity remains ambiguous.

4. Interaction of CaMKII and NMDAR

In addition to the canonical activation mechanism mediated by Ca\(^{2+}\)/calmodulin binding and subsequent autophosphorylation at T286, binding to NMDAR also activates CaMKII (Fig. 2). The interaction between NMDAR subunit GluN2B and CaMKII via the T-site induces autonomous kinase activity of CaMKII, independent of T286 phosphorylation (Bayer et al., 2001). This interaction is crucial for the persistent synaptic translocation of CaMKII that is observed during LTP induction either with chemical LTP or by uncaging caged-glutamate stimulation (Bayer et al., 2006; Bosch et al., 2014; Shen and Meyer, 1999). Subsequent studies support the importance of the CaMKII/NMDAR complex for LTP as well as memory consolidation (Barría and Malinow, 2005; Halt et al., 2012; Incontro et al., 2018; Zhou et al., 2007).

CaMKII T-site-mediated interaction is also important for maintaining basal synaptic transmission. Treating hippocampal slices with tatCN21 at higher concentrations, which competes with the T-site-mediated interaction of CaMKII as well as its kinase activity, has been demonstrated to significantly reduce synaptic transmission (Barcomb et al., 2016; Sanhueza et al., 2011). In GluN2B knock-in mice harboring a L1298A/S1303D mutation, which disrupts the interaction with CaMKII, the effect of tatCN21 was modest, indicating that the effect of tatCN21 is to block the interaction between GluN2B and CaMKII via the T-site (Barcomb et al., 2016). Furthermore, knockout of both CaMKIIα and CaMKIIβ (DKO) via CRISPR genome editing was shown to reduce basal synaptic transmission and LTP (Incontro et al., 2018). The phenotypes in the DKO were rescued by the wild-type, but not by T-site mutation, suggesting that the T-site interaction of CaMKII, perhaps with NMDAR, is critical for both synaptic transmission and LTP. In addition, while a constitutively active mutant of CaMKII, CaMKIIα (CA-CaMKII) increased AMPA-type glutamate receptor (AMPA-R) synaptic currents in DKO neurons, the I205K mutation in CA-CaMKII did not, suggesting that the T-site interaction of CaMKII, perhaps with NMDAR, is essential for LTP (Incontro et al., 2018). The CaMKIIα- GluN2B interaction synthetically localizes CaMKII and maintains its activity locally (Asrican et al., 2007; She et al., 2012). However, it remains unknown whether synthetically localized CaMKII maintains LTP.

5. RAKEC: a model for molecular memory

For the maintenance of LTP, the activity of the molecules in the stimulated synapse has to be increased and sustained. During LTP, the gross activity of CaMKII in a dendritic spine persists for only 1 min after LTP induction (Lee et al., 2009), whereas the enhancement of the synaptic transmission as well as the structural enlargement of the dendritic spine last much longer. To explain this phenomenon, Okamoto et al. (2004) found that actin polymerization (more precisely, the local ratio of F- and G-actin within the stimulated spines) shifted toward F-actin, which was long lasting. F-actin can interact both directly and indirectly with multiple proteins and can serve as a scaffold for synaptic proteins, including surface receptors. Therefore, candidates for the molecule that maintains LTP should be involved in the regulation of the actin cytoskeleton. However, the role of the actin cytoskeleton in LTP maintenance remains controversial. Application of the actin polymerization inhibitor, latrunculin B or cytochalasins, immediately after LTP induction (Krucker et al., 2000), or the actin filament stabilizer, phalloidin, 2 min after LTP induction (Kim and Lisman, 1999) has been demonstrated to reduce LTP. Likewise, Gu et al. (2010) showed that treatment of hippocampal neurons with latrunculin A reduced surface AMPA-R expression, suggesting that F-actin maintains synaptic transmission through AMPA-R, which is a basis for LTP (Gu et al., 2010). Therefore, the regulation of F-actin could be a candidate for LTP maintenance.

Rho family small G proteins are major regulators of actin in a variety of cellular systems (Saneyoshi and Hayashi, 2012). They are bidirectionally controlled by GDP/GTP guanine-nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). GEFs activate GTPase by catalyzing the exchange of bound GDP to GTP, whereas GAPs suppress GTPase by increasing intrinsic GTPase activity. Several lines of evidence indicate that Rho family GTPases, which include RhoA, Cdc42, and Rac1, sit downstream of CaMKII signaling. Using 2pFLIM with specific biosensors, persistent activities were observed during LTP for RhoA, Cdc42 (Murakoshi et al., 2011), and Rac1 (Hedrick et al., 2016; Saneyoshi et al., 2019). These activities have been reported to be...
sensitive to CaMKII inhibitors (Hedrick et al., 2016; Murakoshi et al., 2011; Saneyoshi et al., 2019), suggesting that CaMKII is an upstream target of Rho-GTPases after LTP.

Application of the Rac1 inhibitor, EHT1864 (Shutes et al., 2007), was found to reduce structural LTP even 15 min after LTP induction; thus, activation of Rac1 via the Rac-GEFs is important for LTP maintenance (Saneyoshi et al., 2019). Among the Rac-GEFs known to be regulated under NMDAR signaling (Tolias et al., 2011), Tiam1 (but not Kalirin-7 or betaPIX) forms a stable complex with CaMKII. The CaMKII-Tiam1 complex requires Ca$^{2+}$/calmodulin for binding but, once formed, becomes a Ca$^{2+}$-sensitive independent interaction. A key finding is that the CaMKII-Tiam1 complex acts as molecular machinery to convert the transient Ca$^{2+}$ signal to maintain actin cytoskeleton remodeling. CaMKII binds to Tiam1 when Ca$^{2+}$/calmodulin relieves the autoinhibitory interaction between the catalytic and autoinhibitory domains within CaMKII. As the CaMKII interaction domain within Tiam1 shows high similarity with the autoinhibitory domain of CaMKII (Fig. 3), the CaMKII interaction domain of Tiam1 could be replaced with the autoinhibitory domain of CaMKII. Unlike the CaMKII inhibitor peptide, CaMKINute (Chao et al., 2010), the CaMKII interaction domain in Tiam1 may not block the catalytic domain of CaMKII in the holoenzyme. Thus, it acts as a “pseudo-autoinhibitory” domain for CaMKII to block autoinhibition, which in turn maintains kinase activity (Fig. 4). Similar to the interaction with GluN2B, autonomous activity of CaMKII by Tiam1 is independent of T286 phosphorylation. As CaMKII phosphorylates and activates Tiam1 (Fleming et al., 1999) in the complex with CaMKII, Tiam1 phosphorylation is maintained by activated CaMKII (Saneyoshi et al., 2019). Therefore, both CaMKII and Tiam1 are reciprocally activated, and the activities of both molecules are maintained in the complex. Upon LTP stimulation, phosphorylation and activation of Tiam1 are long lasting, and also stably activate the Rac1 by the complex in dendritic spines (Kojima et al., 2019; Saneyoshi et al., 2019). I proposed a signal complex, such as CaMKII and Tiam1, as a reciprocal activation within a kinase effector complex (RAKEC). Optical activation of PA-AIP2 was shown to be unable to reverse the RAKEC complex with Tiam1 in potentiated synapses (Saneyoshi et al., 2019), probably due to the following reasons: the affinity of AIP2 to CaMKII was too low to disrupt the complex, PA-AIP2 could not reach a high enough concentration by photoactivation at the synapse, or insufficient accessibility of PA-AIP2 to CaMKII in a condensed PSD environment. Together, the RAKEC complex with CaMKII and Tiam1 possess biochemical activities to maintain structure and possibly synaptic function through the actin cytoskeleton in an LTP-specific manner, together with the NMDAR-CaMKII complex to localize CaMKII at the synapses, which may regulate multiple signaling pathways, including Rho-GTPases (Fig. 5).

Although CaMKII is a dominant isoform in the brain tissue, Tiam1 binds not only to CaMKIIα, but also to other isoforms of CaMKII (Saneyoshi et al., 2019). Because CaMKIIβ, another major isoform in the brain tissue, localizes CaMKIIα in the PSD (Borgesus et al., 2011), and performs interactions with actin (Okamoto et al., 2007; O’Leary et al., 2006; Sanabria et al., 2009), CaMKIIβ may play a structural role in targeting the RAKECs of CaMKII in the synapses via actin. Moreover, in contrast to the previous studies that found that a knockout of CaMKIIα had residual LTP, CaMKIIα knock-out mice, such as K42R, T286A, and T305D, showed full blockade of LTP (Giese et al., 1998; Elgersma et al., 2002; Yamagata et al., 2009). Hence, these knock-in CaMKIIα molecules may have a dominant negative effect to form RAKECs in the synapses, as these mutations in CaMKIIα reduced interaction with Tiam1 (Saneyoshi et al., 2019) and GluN2B (O’Leary et al., 2011). Furthermore, CaMKIIα knock-out mice demonstrated compensatory upregulation by CaMKIIβ in the PSD, whereas CaMKIIα knock-in T305D mice did not (Elgersma et al., 2002), suggesting that synaptic localization of CaMKIIα is also regulated by activity-dependent interaction with proteins such as GluN2B or Tiam1 (Fig. 5).

As CaMKII is an oligomeric structure consisting of 12–14 subunits (Bhattacharyya et al., 2016; Myers et al., 2017; Rosenberg et al., 2005), in theory, a single CaMKII holoenzyme can form 12 different RAKECs. Even if two subunits in a holoenzyme are occupied by GluN2B or Tiam1, more than 10 subunits remain free for binding (Fig. 5). Moreover, CaMKII is the most abundant protein in PSD at nearly 100 μM (Otmakhover and Lisman, 2012). There are 2590 CaMKII holoenzymes in the PSD, and 30 are estimated to be bound with GluN2B. Because binding to GluN2B with CaMKII holoenzymes will be through one out of 12 subunits, only ~0.2% of CaMKII is estimated to bind with GluN2B (Feng et al., 2011). Presuming that GluN2B serves in synaptic localization, and Tiam1 is for Rac signaling, it is possible that the other available subunits are for Cdc42 or RhoA activation, because both GTPases are also CaMKII-dependent (Fig. 5) (Murakoshi et al., 2011). This feature can explain why CaMKII is present in such a high concentration in the synapses, in addition to explaining its structural role mediated by the CaMKIIβ subunit-F-actin interaction (Kim et al., 2015, 2016; Okamoto et al., 2007).

CaMKII, therefore, serves as a signaling hub in a center for synaptic plasticity. Since interactions between CaMKII and Tiam1, as well as GluN2B, are activity-dependent, CaMKII could act as an activity-dependent scaffold for assembling signaling proteins in the synapse (Fig. 5). It is of interest to identify CaMKII-associated proteins (Baicum et al., 2015) in the synapses through the T-site and to test if these interacting proteins can form a RAKEC to maintain their biochemical activities. In addition, recent findings have revealed that CaMKII also functions as a factor for survival in neonatal and adult mice (Kool et al., 2019). While the exact mechanism how CaMKII contributes to survival either directly or indirectly is largely unknown, it is of interest to examine possible functions of the RAKEC with CaMKII for survival.

The concept of the RAKEC is based on release from an autoinhibited state to an active state via replacement of the autoinhibitory domain with the pseudo-autoinhibitory domain of its target. As the activity of LTP-related kinases, including CaMKII and ERK, are also persistently activated during LTP (Schmitt et al., 2005; Tang and Yasuda, 2017), these kinases may form a RAKEC with their specific substrates or upstream kinases. In addition, many enzymes utilize autoinhibition in the pseudosubstrate region (Putall and Graves, 2002; Soderling, 1990); therefore, the RAKEC may be a general mechanism for the maintenance of the biochemical activity of kinase and its substrate.

6. Protein lifetime versus molecular memory

There are multiple lines of evidence that support the idea that memories are stored in the brain neuronal circuit and maintained even in pathological brain tissue (Reijmers et al., 2007; Tayler et al., 2013; T286

| Autoinhibitory domain of CaMKII (AID): | ISHRSTVASC--HHRQETVDCKKF |
| CaMKII binding domain in Tiam1: | DSHASRMNQ--LKKQAASAINGG |
| CaMKII binding domain in GluN2B: | NSKAQKKNRKNLREQHSYDTFVDL |
| S1303 |

Fig. 3. Alignment of autoinhibitory domain of CaMKII and CaMKII binding domain from Tiam1 and GluN2B. Human CaMKIIα amino acid 271–293, human Tiam1 amino acid 1541–1565, and human GRIN2B (GluN2B subunit) amino acid 1287–1310 are aligned. Identical amino acids are shown in red, amino acids with similar properties are shown in pink. Two phosphorylation sites by CaMKII are shown: T286 in CaMKII and S1303 in GluN2B.
Han et al., 2009; Liu et al., 2012; Roy et al., 2016; Ryan et al., 2015; Roy et al., 2017; Josselyn and Tonegawa, 2020. The average lifespan of proteins in the hippocampal neurons ranges from a few days to over a week (Camk2a, 6.6 days; GluN2B, 3.45 days; Tiam1, 3.98 days (Dorrbaum et al., 2018); Camk2a, 9.23 days; GluN2B, 6.02 days; Tiam1, 3.97 days (Fornasiero et al., 2018)). Therefore, one important question to be addressed is how molecular memory overcomes protein turnover in the brain tissue.

Although the roles of protein synthesis and transcription in LTP and memory have been extensively studied, the mechanism underlying the process by which newly synthesized proteins are incorporated into specific synapses is not well understood. Recent studies have shown that PSD structure is constructed through a mechanism of liquid-liquid phase separation (LLPS; (Chen et al., 2020; Zeng et al., 2018)). Certain components of the PSD, including GluN2B and PSD-95, are spontaneously phase-separated from other proteins via LLPS. Based on the fact that a component protein in the LLPS can freely go in and out, the newly synthesized PSD proteins at the synapses are likely to be incorporated into the PSD structure via LLPS during LTP.

LLPS is formed by multivalent protein interactions between two proteins (Boeynaems et al., 2018; Li et al., 2012). As the CaMKII holoenzyme consists of 12–14 subunits through its association domains as well as the autoinhibitory T-site interaction in a catalytic domain, the interaction of CaMKII with synaptic proteins, including NMDAR, could be phase-separated from other synaptic proteins. Tiam1 is another multi-domain protein that interacts with many proteins, including NMDAR (Tolias et al., 2005), Ras (Lambert et al., 2002), and EphB (Tanaka et al., 2004; Um et al., 2014). The RAKEC with CaMKII and Tiam1 is likely to overcome protein turnover through the feature of LLPS in the stimulated synapse. Moreover, synaptic localization of βPIX may also be regulated by LLPS (Zhu et al., 2020). Zhu et al. (2020) found that βPIX can be incorporated into the PSD via LLPS during LTP.

In the stimulated synapse, CaMKII and Tiam1 regulate Rac to actin cytoskeleton signaling. Other RAKECs are likely to serve as different signaling cascades, such as small GTPases. CaMKII may also act as an activity-dependent scaffold to assemble proteins at the synapse in addition to F-actin binding through CaMKIIβ.

Fig. 4. The RAKEC between CaMKII and Tiam1. (Left) CaMKII is activated by Ca2+/calmodulin. Calmodulin-activated CaMKII autophosphorylates on T286, which generates autonomous activity. When T286 phosphorylation is removed, the activity of CaMKII returns to basal level. (Right) Activated CaMKII binds to Tiam1, and forms reciprocal activation within a kinase effector complex (RAKEC). In the RAKEC, CaMKII and Tiam1 reciprocally activate each other to maintain its activity even in the absence of T286 phosphorylation. The RAKEC stores the biochemical information as molecular memory. Active CaMKII are shown in the brighter colors. CaM, Ca2+/calmodulin; P represents phosphorylation.
7. Conclusions and future directions

In this review, I propose a novel mechanism for the RAKE complex that serves as a molecular memory in the brain tissue. Tiam1 is probably just one of many molecules that retain their activity during LTP. RAKE complex, in combination with the liquid-liquid phase separation-mediated principle of protein accumulation and turnover at the synapse, explains the persistent functional and structural changes in dendritic spines after LTP induction. Along with the expanding optogenetic toolbox (Gautier et al., 2014), improvements in resolution of time and space as well as greater efficiency to manipulate the plasticity-related molecules in neurons (Okuda et al., 2020) will allow more in-depth investigation of these molecules in the future. Thus, using more advanced optical tools and reconstitution approaches together with newer concepts such as the RAREC or LLP5, further research is required to understand the synapse-specific molecular memory in vitro and in vivo.

CRediT authorship contribution statement

Takeo Saneyoshi: Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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