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PROGRESS

How does PKMζ maintain long-term memory?

Todd C. Sacktor

Abstract | Most of the molecular mechanisms contributing to long-term memory have been found to consolidate information within a brief time window after learning, but not to maintain information during memory storage. However, with the discovery that synaptic long-term potentiation is maintained by the persistently active protein kinase, protein kinase Mζ (PKMζ), a possible mechanism of memory storage has been identified. Recent research shows how PKMζ might perpetuate information both at synapses and during long-term memory.

Since the beginning of experimental neurobiology, scientists have searched for the physical substrate of long-term memory storage (the memory 'trace'). In the 1970s and 1980s, experiments in invertebrate model systems, such as *Drosophila melanogaster* and *Aplysia californica*, provided compelling data to show that short-term memory is mediated by transient post-translational modifications, particularly phosphorylation by protein kinases[1,2]. These modifications affect the function of synaptic proteins, briefly altering the strength of the connections within networks of neurons that control behaviour. Although the specific content of a given memory depends on the underlying neuronal network in all its complexity, these pioneering studies suggested that there might be a fundamental simplicity to the molecular mechanisms of memory.

Inspired by this success, neuroscientists discovered scores of molecules in the 1990s that were important for the formation of long-term memory and persistent forms of synaptic plasticity, such as long-term potentiation (LTP)[3]. However, the physical substrates of the long-term memory trace remained an enigma. This was because the molecules discovered were important for forming long-term memory, but not for maintaining memory. Neurotransmitter receptors (*N*-methyl-D-aspartic acid receptors (NMDARs) and dopamine receptors), second messengers and their effectors (Ca^{2+}, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), mitogen-activated protein kinase (MAPK) and protein kinase A (PKA)), and growth and transcription factors (brain-derived neurotrophic factor (BDNF) and cyclic AMP-responsive element-binding protein (CREB)), were found to act during — or for a few minutes to hours after — learning, in the processes of memory encoding or cellular memory consolidation. Many of the signalling molecules involved in this initial stabilization of memory were found to regulate new protein synthesis. Thus, gene expression became the hallmark of memory consolidation (and, as subsequently shown, of reconsolidation if the memory had recently been retrieved and re-encoded[4]). However, when inhibitors of these molecules were given after this initial time window to behaviourally conditioned rodents and *A. californica*, none of the agents disrupted the storage of an established long-term memory. Similarly, many inhibitors that blocked the induction of the protein synthesis-dependent late phase of LTP in hippocampal slices did not reverse the maintenance of the potentiation when applied 1–2 hours after induction[3]. Thus, by the beginning of the twenty-first century, it was generally believed by researchers in the learning and memory field that the memory trace was maintained not by the persistent signalling of molecules, but in the morphology of synaptic connections[5]. Because new or remodelled synapses were presumed to share the same molecules as synapses formed during development, this hypothesis seemed to explain why long-term memories could not be erased.

In the past few years, however, a candidate, persistent enzymatic molecular mechanism for the long-term memory trace has emerged. The main molecule involved in this mechanism is a constitutively active protein kinase C (PKC) isoform, protein kinase Mζ (PKMζ), which is expressed exclusively in neural tissue and enriched in the forebrain[6,7]. This enzyme perpetuates both LTP maintenance and the long-term memory trace through continual phosphorylation that persistently enhances postsynaptic AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) responses, which mediate fast excitatory synaptic transmission in the brain[7]. Late-LTP maintenance is reversed by inhibiting PKMζ, even when inhibitors are applied hours to days after LTP induction[8–12], and several forms of long-term memory are rapidly erased by locally inhibiting PKMζ in different brain regions of rats and mice, from days to even weeks and months after training[11–20] (see Supplementary information S1 (table)). PKMζ function seems to be evolutionarily conserved: inhibiting the *A. californica* homologue of PKMζ erases established behavioural long-term sensitization[21] and its underlying synaptic plasticity, long-term facilitation[22], and the *D. melanogaster* homologue is crucial for persistent, classically conditioned olfactory memory in the fly[23].

For the first time, therefore, neuroscientists have experimental evidence for the storage mechanism of a long-term memory trace. But this persistent enzymatic mechanism of memory storage raises new questions, some of which were anticipated when Francis Crick first proposed that enzymes might perpetuate memory[24], and in the subsequent early attempts to model persistent kinases in the 1980s[25–27]. First, as the activation of most protein kinases lasts only seconds to minutes, how can the activation of PKMζ be maintained for weeks to months? Second, how does only a brief exposure to a PKMζ inhibitor rapidly disrupt a stable memory? The disruption of memory by PKMζ inhibition seems to be permanent as there is no spontaneous recovery even weeks after the disruption. Yet, after the inhibitors are removed, new memories can be learned and stored with retraining[11,13,15,17]. So, third, how does transiently inhibiting PKMζ produce persistent retrograde memory erasure, with no anterograde effect? Here, I discuss several recent papers that provide insights into these fundamental issues.

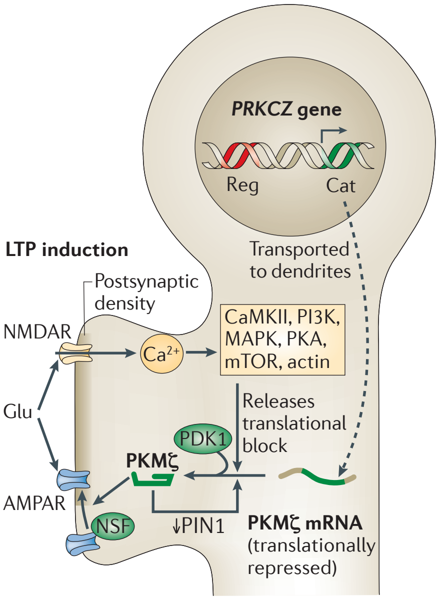

Figure 1 | **PKMζ formation in LTP.** The protein kinase C, zeta (*PRKCZ*) gene has two promoters, one producing a full-length protein kinase Cζ (PKCζ) from exons encoding a regulatory domain (Reg; shown in red) and a catalytic domain (Cat; shown in green). In neurons, an internal promoter produces a protein kinase Mζ (PKMζ) mRNA that encodes a ζ catalytic domain without a regulatory domain. The PKMζ mRNA is transported to dendrites and is translationally repressed by PIN1 (protein interacting with NIMA1). During long-term potentiation induction, multiple signalling pathways stimulated by NMDAR (*N*-methyl-D-aspartic acid receptor) activation are required to release the translational block. Once synthesized, PKMζ binds to and is phosphorylated by phosphoinositide-dependent protein kinase 1 (PDK1), which increases the constitutive kinase activity of PKMζ. PKMζ then initiates a positive feedback loop through inhibition of PIN1 to maintain increased dendritic translation of the PKMζ message. PKMζ potentiates AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) responses by increasing the number of the receptors in the postsynaptic density through the action of the trafficking protein N-ethylmaleimide-sensitive factor (NSF). CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; Glu, glutamate; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A.

How is PKMζ activity maintained?

During LTP induction and memory formation, postsynaptic NMDAR activation causes a rise in Ca^{2+}. This triggers a cascade of second messengers that activate protein kinases and other effector molecules[3]. As the second messengers are rapidly eliminated, the activities of most of the effectors fade within minutes. By contrast, once PKMζ is formed, its activity persists. This unique feature of PKMζ comes from the unusual structure of the enzyme as a second messenger-independent, constitutively active isoform of PKC[6,28].

PKMζ structure and function. Most PKC isoforms consist of an amino-terminal regulatory domain and a carboxy-terminal catalytic domain[29]. The regulatory domain contains second messenger-binding sites and an autoinhibitory pseudosubstrate, which interacts with and inhibits the catalytic domain. Second messengers, such as diacylglycerol, or Ca^{2+} for some isoforms, bind to the regulatory domain and produce a conformational change that releases the autoinhibition of the pseudosubstrate, activating the kinase. When the second messengers are metabolized, PKC folds back into its inactive conformation.

PKMζ is activated differently from other PKC isoforms (FIG. 1). In the brain, transcription from an internal promoter within the protein kinase C, zeta (*PRKCZ)* gene produces a PKMζ mRNA that encodes a ζ catalytic domain without a regulatory domain. Lacking the regulatory domain's autoinhibition, this catalytic domain is constitutively, and thus persistently, active[6]. The PKMζ mRNA is transported to dendrites of neurons[30], and under basal conditions is translationally repressed by its long 5′-untranslated region[6]. During LTP induction by NMDAR activation in the postsynaptic density, CaMKII, phosphatidylinositol 3-kinase (PI3K), MAPK, PKA, mammalian target of rapamycin (mTOR) and actin filament formation are stimulated. All these signalling molecules are required to release the translational block on PKMζ synthesis[31,32]. Immediately after translation, PKMζ has low levels of activity until it binds to another kinase, phosphoinositide-dependent protein kinase 1 (PDK1), which phosphorylates PKMζ and converts it into a conformation with high constitutive activity[32]. PKMζ is thus both the site of convergence of many signals in LTP induction and the source of persistent phosphorylation in LTP maintenance.

Because of its constitutive activity, persistent increases in the amount of PKMζ would result in persistent increases in kinase activity[28,33]. But after its initial synthesis, how can increased amounts of PKMζ persist despite the turnover of individual PKMζ molecules? Indeed, even memories that are months old still depend on persistent PKMζ activity[11,15,20]. Increased ζ mRNA levels have been observed in the rat hippocampus after training in the watermaze[34] but, because the PKMζ message is translationally repressed, a mechanism to persistently increase translation of the message might still be required to maintain the increased amounts of the PKMζ protein that are required for storing memory.

Persistent translation of PKMζ. In 2010, a signalling pathway was identified that acts in a positive feedback loop to maintain increased amounts of PKMζ through persistently increased translation[35] (FIG. 1). The translation of messages transported to the dendrites of neurons, including PKMζ mRNA, is suppressed by the action of PIN1 (protein interacting with NIMA1), a prolyl isomerase. Glutamate signalling, as occurs in LTP induction, decreases PIN1 activity, releasing its repression and allowing PKMζ synthesis. Once synthesized, PKMζ phosphorylates and inhibits PIN1, so sustaining PKMζ synthesis. Thus, the local translation of PKMζ may be self-perpetuating, maintaining high levels of the kinase at appropriate synapses[36]. This localized persistent increase in PKMζ continually reconfigures the distribution of AMPARs through the interaction between the trafficking protein N-ethylmaleimide-sensitive factor (NSF) and the glutamate receptor 2 (GluR2; also known as GluA2 and GluRB) subunit of the AMPAR to maintain increased numbers of receptors at postsynaptic sites, potentiating synaptic transmission[37].

Other mechanisms for prolonging the translation of PKMζ may also contribute to memory persistence. The *A. californica* homologue of the translation factor cytoplasmic polyadenylation element-binding protein (CPEB)[38,39] sustains the persistence of protein synthesis-dependent memory in this model system[40]. A neuronal isoform of CPEB contains an N-terminal domain that confers on the protein self-perpetuating, prion-like properties[38]. This *A. californica* CPEB can exist in two conformations, one of which can convert the other into its own conformational state. Unlike other prion proteins, the dominant conformation of CPEB is the more active, suggesting a mechanism for the persistence of increased translation. Because the homologue of PKMζ maintains long-term memory in

A. californica for at least a week[21], CPEB may help prolong PKMζ synthesis, and thus the two mechanisms of persistence might work together to sustain memory. This notion is further supported by evidence in *D. melanogaster* that the CPEB homologue ORB2 targets the mRNA of atypical PKC[41] that is crucial for memory persistence in the fly[23].

Brief applications of protein synthesis inhibitors to hippocampal slices or to behaving animals can block LTP induction and long-term memory formation. However, they do not disrupt LTP maintenance or long-term memory that persists a day or more in rodents and *D. melanogaster*[42] or more than two days in *A. california*[40]. This is consistent with a PKMζ half-life that is much longer than the few hours of protein synthesis inhibition produced by commonly used translation inhibitors, such as anisomycin[33]. By contrast, in as little as 2 hours, applications of exogenous PKMζ kinase inhibitors — the pseudosubstrate zeta inhibitory peptide (ZIP) and the PKC catalytic domain inhibitor chelerythrine — disrupt hippocampal LTP maintenance both in rat brain slices[8–10] and in the rat and mouse *in vivo*[11,12]. In rat, mouse and *A. californica*, these inhibitors also disrupt long-term memories that can be from 1 day to months old[11–21] (see Supplementary information S1 (table)). Exogenous overexpression in the rat insular cortex of a dominant negative form of PKMζ that reduces PKMζ activity also disrupts the established memory underlying conditioned taste aversion (R. Shema, T.C.S. and Y. Dudai, unpublished observations). How can LTP maintenance and memories that were stable become so fragile with the loss of PKMζ activity? The answer may lie in how PKMζ potentiates AMPAR-mediated synaptic transmission.

How does PKMζ maintain memory?

Reconfiguring postsynaptic AMPAR trafficking. In CA1 pyramidal cells recorded in hippocampal slices, the postsynaptic perfusion of PKMζ potentiates synaptic transmission by reconfiguring the trafficking of AMPARs to persistently increase their number at postsynaptic sites[8,37,43] (FIG. 2). Although the site of phosphorylation is unknown, PKMζ acts through the GluR2 subunit, which forms heteromeric AMPARs with either GluR1 or GluR3 at mature CA3–CA1 pyramidal cell synapses. During low-frequency synaptic transmission[44,45], interactions between GluR2 and the trafficking protein NSF maintain basal numbers of postsynaptic GluR2-containing AMPARs. This is evidenced by a gradual

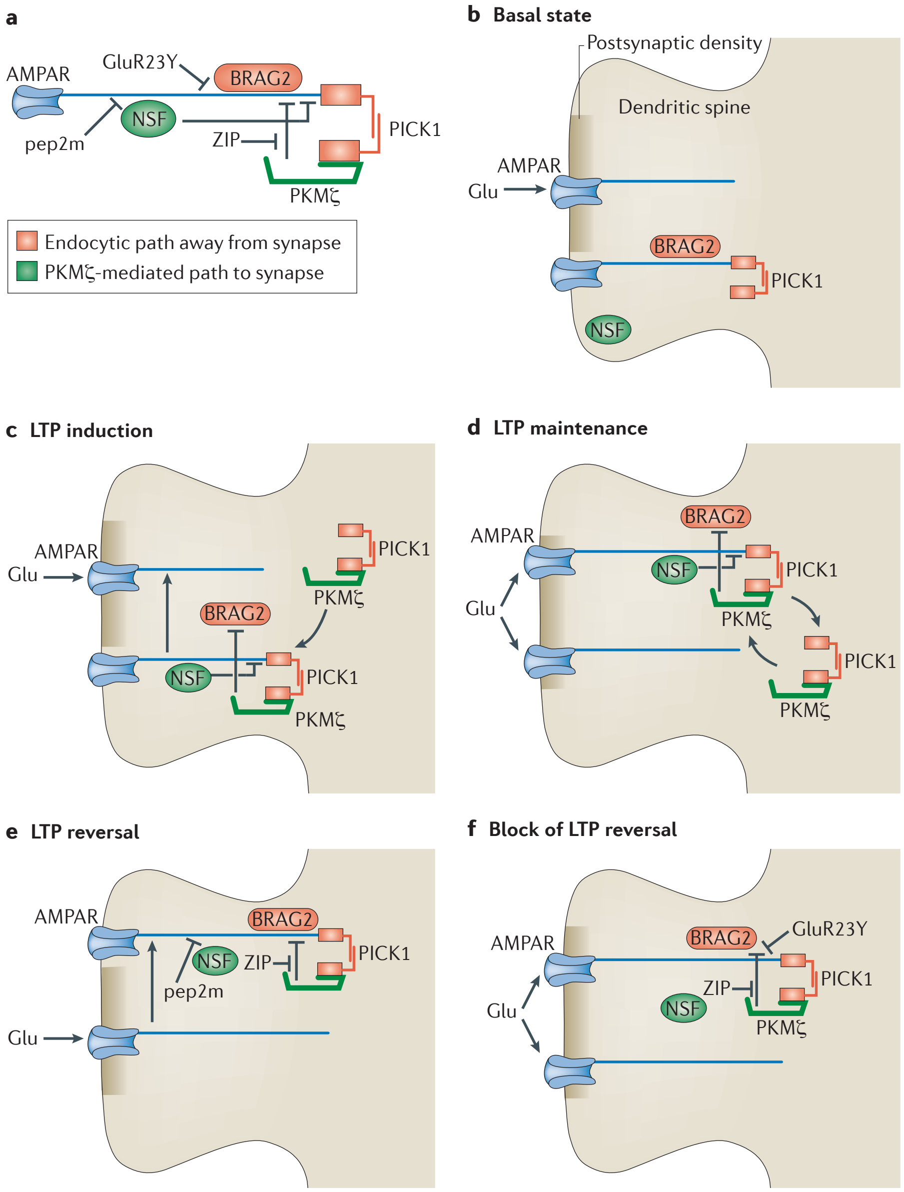

Figure 2 | **Mechanism of synaptic potentiation by PKMζ in LTP maintenance. a** | The carboxy-terminal of the glutamate receptor 2 subunit (GluR2) of the AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) binds to proteins that traffic the receptor to postsynaptic sites (protein kinase Mζ (PKMζ) and N-ethylmaleimide-sensitive factor (NSF)) or away from postsynaptic sites (protein interacting with C kinase 1 (PICK1) dimers, brefeldin resistant Arf-GEF 2 protein (BRAG2; also known as IQSEC1), Arf6 and adaptor protein 2 (not shown)). Agents that block the trafficking to the synapse — such as zeta inhibitory peptide (ZIP), which inhibits PKMζ, and pep2m, which blocks NSF binding to GluR2 — both prevent and reverse long-term potentiation (LTP) maintenance. GluR23Y, which blocks BRAG2 binding to GluR2, prevents the reversal of LTP by ZIP. **b** | In the basal state, constitutive endocytosis maintains GluR2-containing AMPARs in a pool held outside the synapse by PICK1. **c** | In LTP induction, newly synthesized PKMζ binds to PICK1 dimers, and PKMζ phosphorylates a substrate, possibly the AMPAR C-terminal or associated protein, which decreases AMPAR endocytosis and increases the action of NSF, which disrupts AMPARs from PICK1. The receptors traffic to and bind proteins in the postsynaptic density, potentiating synaptic transmission. **d** | In LTP maintenance, PKMζ continues to decrease receptor endocytosis and to enhance the action of NSF that prevents PICK1-mediated postsynaptic removal of GluR2, thus stabilizing the increased number of receptors at postsynaptic sites. **e** | LTP reversal occurs when ZIP blocks PKMζ activity, increasing receptor endocytosis and decreasing NSF efficacy so that it cannot release GluR2 from PICK1. BRAG2 and PICK1 initiate endocytosis that removes GluR2 from the synapse. Pep2m reverses LTP maintenance downstream of PKMζ action by blocking the interaction of NSF and the GluR2 C-terminal. **f** | GluR23Y, which inhibits binding of BRAG2 to the GluR2 C-terminal, prevents the endocytic pathway from removing AMPARs, thus blocking the reversal of LTP by ZIP.

reduction of AMPAR responses when this interaction is blocked by the postsynaptic perfusion of a peptide, termed pep2m, that mimics the binding site of NSF in the middle of the C-terminal end of GluR2 (REFS 44–48) (FIG. 2a). This action of NSF may occur through the ability of NSF to disrupt the interaction between the C-terminal end of GluR2 and the PDZ domain-containing protein PICK1 (protein interacting with C kinase 1), a homodimer that participates in the endocytic removal of AMPARs from synapses[49]. Thus, GluR2–NSF interactions prevent a long-term depression (LTD)-like decrease during basal synaptic transmission, stabilizing the number of postsynaptic AMPARs.

PKMζ transforms this mechanism of postsynaptic AMPAR homeostasis into a mechanism of synaptic potentiation. In addition to GluR2-containing AMPARs at the synapse, a pool of these receptors is maintained outside the synapse by binding to PICK1 (FIG. 2b). Release of these receptors from PICK1 potentiates synaptic transmission in hippocampal pyramidal cells, as observed when the interaction between GluR2 and PICK1 is disrupted by postsynaptic perfusion of a peptide that mimics the C-terminal of the receptor and competes for the PDZ domain in PICK1 (REFS 37,50–52). The synaptic potentiation produced by this peptide mimics and occludes the potentiation caused by postsynaptic perfusion of PKMζ. Conversely, the potentiation by PKMζ is blocked by pep2m and other NSF inhibitors that would prevent the release of GluR2 from PICK1 (REF. 37). Although the mechanism of the interaction between PKMζ and NSF is not yet known, this suggests that PKMζ, which also forms a complex with PICK1 (REF. 37), functionally enhances the ability of NSF to release GluR2-containing receptors from the PICK1-bound extrasynaptic pool, thereby inducing LTP (FIG. 2c). In addition, a cell-permeant form of pep2m that also blocks PKMζ-mediated AMPAR potentiation not only prevents but reverses late-LTP maintenance[37] — the only agent other than PKMζ kinase inhibitors known to have this effect. This indicates that the persistent action of PKMζ continually requires GluR2–NSF interactions to maintain LTP (FIG. 2d,e).

An active opposition. Once PKMζ drives AMPARs to the synapse in LTP induction, why is the kinase necessary to maintain synaptic potentiation? A recent paper indicates that when PKMζ traffics AMPARs to the synapse, homeostatic responses are activated that tend to drive the receptors back out and return the synapse to its pre-potentiated state[17] (FIG. 2a,e). Thus, LTP maintenance involves a continual battle between PKMζ and homeostatic mechanisms over the location of AMPARs — a battle that is persistently won by PKMζ. However, when PKMζ inhibitors, such as ZIP, are applied experimentally, the additional postsynaptic AMPARs are actively eliminated and the synapse returns to its naive, basal state[8,9,11,12].

The mechanism driving AMPARs out of the synapse during ZIP-mediated depotentiation is closely related to that seen during LTD[17] (FIG. 2a,e). A tyrosine-rich region adjacent to the NSF-binding site in the GluR2 C-terminal is critical for the endocytosis and elimination of postsynaptic GluR2-containing AMPARs in both NMDAR- and metabotropic glutamate receptor (mGluR)-dependent LTD[53–55] and during ZIP-mediated depotentiation[17]. A recent paper has shown this tyrosine-rich region binds to the guanine-nucleotide exchange factor brefeldin-resistant Arf-GEF 2 protein (BRAG2; also known as IQSEC1), which activates the GTPase Arf6 (REF. 55), which then recruits adaptor protein complex 2 (AP2), a key mediator of endocytosis at the plasma membrane. AP2 also binds the GluR2 C-terminal at a site overlapping the binding site of NSF[56], and thus may compete for binding. A peptide called GluR23Y, which mimics the tyrosine rich-region of the GluR2 C-terminal[53] (FIG. 2a), prevents increases in AMPAR endocytosis induced by insulin and activity-dependent LTD[53,54], presumably by blocking BRAG2 binding.

Postsynaptic perfusion of GluR23Y also prevents the ability of ZIP to reverse LTP maintenance[17] (FIG. 2e,f). Similarly, 1 day after fear conditioning in rats, injecting a cell-permeant GluR23Y peptide into the basolateral amygdala 1 hour before injecting ZIP prevents both the amnesia and the loss of GluR2 in postsynaptic density fractions that are seen after injection of the PKMζ inhibitor alone[17]. Identical behavioural results were demonstrated for object location memory when the injections were made into the dorsal hippocampus[17]. Previously, GluR2 had been implicated in PKMζ-mediated synaptic potentiation through inhibiting the actions of NSF and PICK1, and not by the use of ZIP. Therefore, in addition to revealing the underlying battle over GluR2-containing AMPARs during LTP and memory persistence, these more recent experiments also demonstrate that ZIP specifically targets the action of PKMζ on these receptors both in brain slices and *in vivo*.

Interestingly, although ZIP decreased GluR2 in the postsynaptic density fractions of the basolateral amygdala in fear-conditioned animals, this peptide inhibitor of PKMζ had no effect on GluR2 in fractions from the same region of the brain in untrained animals[17]. This is analogous to the ability of ZIP to reduce AMPAR responses at potentiated synapses, but not at non-potentiated synapses[8–12]. Recently, increases in synaptic transmission have been observed *in vivo* at CA3–CA1 synapses of rats and mice after training on hippocampus-dependent tasks[12,57,58], with persistent increases sustained for at least 1 day after the last training session during trace eye-blink conditioning[12,58]. ZIP reverses this persistent increased synaptic transmission in conditioned animals but, as expected, does not affect synaptic transmission in unconditioned animals[12]. These results suggest that the persistent action of PKMζ is specific to synapses storing experience-dependent information, but has no lasting role in the basal synaptic transmission of neural circuitry that is established during development. Thus, the information stored in a long-term memory trace appears to depend on the presence or absence of PKMζ at specific synapses. How then is PKMζ maintained at specific synapses during memory storage? Although we do not yet know the answer, insight may be gained by understanding how synaptic information maintained by PKMζ is erased.

How can memory be erased?

If information is encoded as the presence or absence of PKMζ at specific synapses, and interrupting the activity of PKMζ effectively erases this information, then the persistent activity of PKMζ itself might maintain the kinase at appropriate synaptic sites — a form of PKMζ synaptic 'autotagging'. PKMζ may maintain its synapse-specific compartmentalization by a mechanism distinct from that by which it drives AMPARs to the synapse, but the simplest hypothesis is that these two functions of PKMζ are related.

A model of PKMζ synaptic autotagging. During memory induction, PKMζ is synthesized and captured at recently activated synapses that have undergone synaptic tagging[10], perhaps by binding to PICK1 dimers with which it forms a complex[37] (FIG. 3a). PKMζ phosphorylates a substrate, possibly the GluR2 C-terminal or its associated proteins, resulting in the release of the receptors from PICK1 by NSF and the redistribution of the

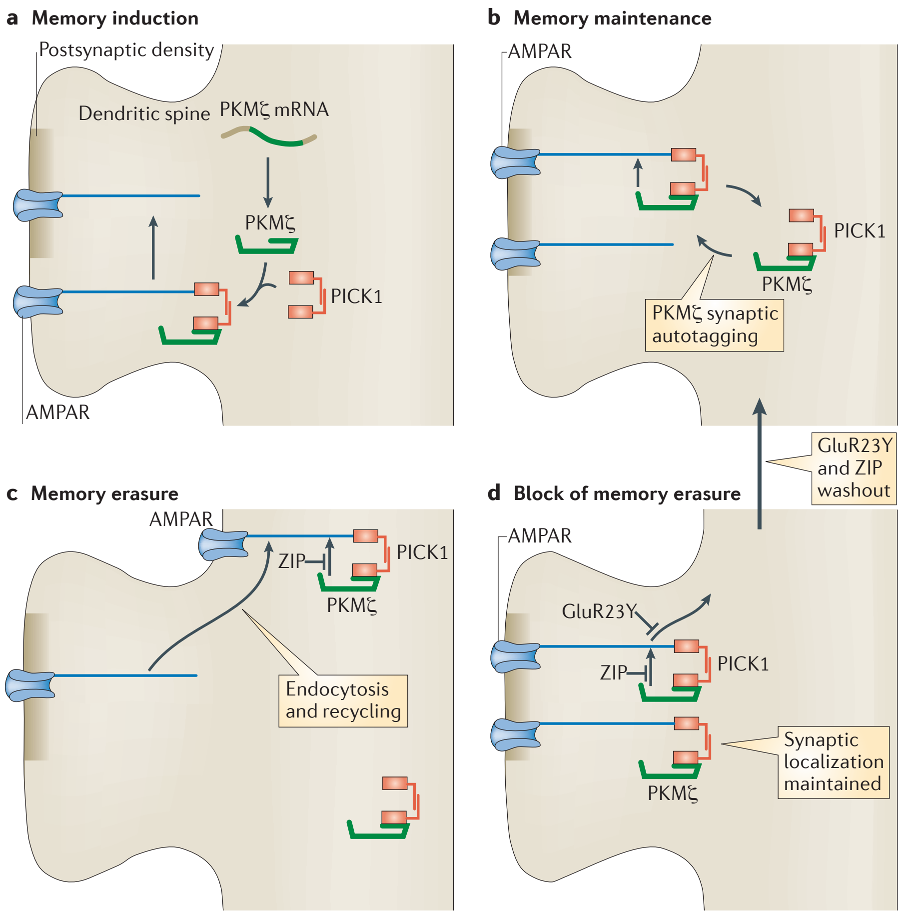

Figure 3 | **Model of PKMζ synaptic autotagging in memory maintenance. a** | In memory induction, protein kinase Mζ (PKMζ) is locally synthesized and captured at activated synapses by PICK1 (protein interacting with C kinase 1). The PKMζ drives extrasynaptic AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors) to the synapse, potentiating synaptic transmission. **b** | In memory maintenance, the increased number of postsynaptic AMPARs forms a tag that maintains PKMζ at potentiated synapses. PKMζ kinase activity stabilizes AMPARs at synaptic sites, and after dephosphorylation by phosphatases, the free carboxy-terminal of the glutamate receptor 2 subunit (GluR2) of the AMPAR acts as a tag that captures and maintains PKMζ–PICK1 complexes at the potentiated synapse. Thus, PKMζ activity maintains both synaptic potentiation and the location of the kinase at the potentiated synapse. **c** | Zeta inhibitory peptide (ZIP) blocks PKMζ activity, breaking the synaptic autotagging cycle. Both the GluR2-containing AMPAR and the PKMζ are removed from the synapse by endocytosis and recycling to extrasynaptic membrane. The information as to which synapse had contained PKMζ is permanently lost, and memory is erased. **d** | Application of GluR23Y before ZIP prevents the endocytic removal of the AMPAR and blocks memory loss. After both drugs are eliminated (shown by the arrow from part **d** back to part **b**), the PKMζ, which remains at the synapse through interaction with PICK1 and the postsynaptic GluR2, resumes synaptic autotagging.

extrasynaptic receptors to postsynaptic sites to initiate LTP[37].

During memory maintenance, the increased amount of GluR2 at the potentiated synapse acts as a ‘tag’ that captures the PKMζ–PICK1 complex (FIG. 3b). After PKMζ phosphorylation has driven extrasynaptic GluR2-containing AMPARs to the synapse and NSF has released the PKMζ–PICK1 complex from the GluR2 C-terminals, phosphatases would tend to reverse this process, thereby reducing NSF action and initiating the endocytic pathway that would eliminate the increased numbers of receptors from the synapse. But the free GluR2 C-terminals at the synapse also reconstitute a synaptic tag that recaptures PKMζ–PICK1. Rephosphorylation by PKMζ then blocks the endocytic pathway, stabilizing the receptors at the synapse. Through this cycle of phosphorylation and dephosphorylation, the persistent activity of PKMζ maintains increased levels of both AMPARs and itself at potentiated synapses.

During LTP reversal or memory erasure by ZIP, PKMζ activity is inhibited, breaking the cycle, and this allows the endocytic pathway to remove the extra receptors from the synapse (FIG. 3c). (During LTP reversal by pep2m, NSF binding to the GluR2 C-terminal is blocked, breaking the cycle downstream of PKMζ[37].) After endocytosis, the AMPAR and the inhibited PKMζ, which may remain together through PICK1, traffic away from the synapse, and the receptors can recycle back to the extrasynaptic plasma membrane. Thus, in this model, both the synaptic potentiation by PKMζ and the synapse-specific compartmentalization of PKMζ are lost when the kinase is inhibited. Even after ZIP is eliminated, the information encoded as which synapses originally contained PKMζ cannot be recovered.

Conversely, in the case of overexpression of PKMζ, the PKMζ synaptic autotagging model predicts that, if the amount of overexpression does not saturate all synapses, the exogenously expressed PKMζ might be selectively captured by the increased amount of AMPARs at synapses potentiated by endogenous PKMζ. Thus, PKMζ overexpression could in theory produce an enhancement of old, weak memories.

Evidence for the model. Is there evidence for a link between AMPAR trafficking and the persistence of PKMζ-mediated memory storage? The model predicts that if the removal of GluR2-containing AMPARs from the synapse were prevented, the inhibition of PKMζ would not disrupt memory storage. In the experiments with GluR23Y, blocking AMPAR endocytosis prevented the disruption of memory expression by ZIP[17], as discussed above. But what happens to the memory a week later, when both GluR23Y and ZIP have been eliminated? If the action of PKMζ on AMPAR trafficking is separate from its capacity to maintain itself at specific synapses, then when GluR23Y and ZIP are eliminated, memory loss should occur. This is because the PKMζ-mediated increase in postsynaptic AMPARs would have been transiently preserved by the GluR23Y, but the synaptic localization of PKMζ would have been permanently disrupted by ZIP. By contrast, if PKMζ-mediated AMPAR trafficking and PKMζ synaptic autotagging are closely related, the memory would persist, because blocking AMPAR removal by GluR23Y would also preserve PKMζ at the appropriate synapses (FIG. 3d), allowing the PKMζ synaptic autotagging to recover after drug wash-out (FIG. 3d to 3b). The answer is that even 10 days after GluR23Y and ZIP injections in the basolateral amygdala, fear conditioning memory is preserved[17]. This indicates that trafficking of GluR2-containing AMPARs is crucial not only for the expression of memory downstream of PKMζ, but also for the persistence of memory storage by PKMζ.

Future perspectives

With the discovery of the role of PKMζ in the long-term memory trace, many new questions and research opportunities arise that did not exist just a few years ago. We need to understand more about the substrates of PKMζ phosphorylation that mediate synaptic potentiation, and the mechanisms, both translational and post-translational, that maintain appropriate amounts of the kinase at specific synapses during memory maintenance. We do not yet understand how new information might be incorporated into PKMζ-mediated memory traces[59] by reconsolidation[4] or during sleep[60]. If the molecular mechanism of memory storage can be reduced to the presence or absence of PKMζ at specific synapses, can we quantify a memory trace by counting the number of dendritic spines containing PKMζ after an animal learns and remembers a task? What prevents multiple memories from saturating all the synapses of the brain with PKMζ? Likewise, what makes the encoding of memories in rats and mice raised in laboratory environments so sparse, such that ZIP does not affect their basal synaptic transmission when there has been no experimental training? Perhaps the active elimination of PKMζ to reduce redundancy of information storage is a role for long-term depression (LTD), a form of plasticity that degrades PKMζ[61].

Glossary

Cellular memory consolidation
The molecular mechanisms that convert memories into an enduring form. The process typically lasts for a few hours after learning and is associated with new protein synthesis. It is distinct from systems memory consolidation, which involves shifts in the neuronal circuitry that subserves a memory and can take weeks or longer.

Long-term memory storage
The physiological mechanism in the brain that perpetuates enduring memories. The storage phase of long-term memory begins from a few hours to a day after learning and can last a lifetime.

Long-term potentiation
A persistent enhancement of excitatory synaptic transmission lasting hours to days, triggered by strong, typically high-frequency, afferent stimulation of the synapse. It is widely studied as a putative physiological basis of long-term memory.

PDZ domain
A common protein structural motif that interacts with specific carboxy-terminal sequences of other proteins. The intracellular distribution and trafficking of many proteins are regulated by their binding to PDZ domain-containing proteins.

Postsynaptic density
A cytoskeletal specialization of the synapse identified by electron microscopy as an electron-dense region at the membrane of the postsynaptic neuron. It concentrates and organizes neurotransmitter receptors, receptor-binding proteins and postsynaptic signalling molecules.

Synaptic tagging
A hypothesis to explain the potentiation during late-LTP (long-term potentiation) of activated synapses by proteins newly synthesized in the neuronal cell body or dendrite. Afferent stimulation sets up a ‘tag’ specifically at activated synapses that captures the newly synthesized plasticity-related proteins.

Trace eye-blink conditioning
A form of classical conditioning in which the conditioned stimulus (CS; typically an auditory or visual stimulus) precedes the unconditioned stimulus (US; an eye-blink-eliciting stimulus such as a puff of air to the cornea) by a stimulus-free period (trace interval). Trace eye-blink conditioning requires both an intact cerebellum and hippocampus.

Although there is much more to learn, recent progress has already brought many surprises. We now know that signalling molecules are the driving force of information storage, not just information consolidation both at synapses and during behaviour, and that a memory trace can be erased without damaging the circuitry of the brain. We know that this driving force of information storage is an active enzymatic process continually resisting a counterbalancing enzymatic mechanism for erasing information, which would drive synapses to their naive state, and the brain rapidly to a blank slate. It may not be surprising that the process of acquiring and maintaining knowledge needs energy and a mechanism of persistence, but to see this manifest in a persistently active enzyme is a considerable advance in our understanding of how memories are formed and stored.

Todd C. Sacktor is at The Robert F. Furchgott Center of Neural and Behavioural Science, Departments of Physiology and Pharmacology and of Neurology, State University of New York (SUNY) Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York 11203, USA.

e-mail: tsacktor@downstate.edu

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Competing interests statement

The author declares no competing financial interests.

FURTHER INFORMATION

Todd C. Sacktor's homepage: http://www.downstate.edu/pharmacology/faculty/sacktor.html

SUPPLEMENTARY INFORMATION

See online article: S1 (table)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

Supplementary information S1 | **Consolidated long-term memories disrupted by PKMζ inhibition**

Behaviour	**Injection site**	**Species**	**Reference**
Active place avoidance	DH	Rat	1,2
Water maze	DH	Rat	2
Radial arm maze	DH	Rat	2
Novel object location	DH	Rat	3,4
Trace eye-blink conditioning	DH	Mouse	5
Contextual fear conditioning	BLA	Rat	2,6
Auditory fear conditioning	BLA	Rat	2,3,6
Inhibitory avoidance	BLA	Rat	2
Conditioned taste aversion	IN	Rat	7,8
Skilled motor learning	SM	Rat	9
Auditory fear conditioning (remote memory)	secAC	Rat	10
Visual fear conditioning (remote memory)	secOC	Rat	10
Olfactory fear conditioning (remote memory)	PC	Rat	10
Long-term sensitization	CNS	*A. californica*	11

DH, dorsal hippocampus; BLA, basolateral amygdala; IN, insular cortex; SM,sensorimotor cortex; secAC, secondary auditory cortex; secOC, secondary occipital cortex; PC, piriform cortex; CNS, central nervous system

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