The genetics of PKMζ and memory maintenance

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Elucidating the molecular mechanisms that maintain long-term memory is a fundamental goal of neuroscience. Accumulating evidence suggests that persistent signaling by the atypical protein kinase C (PKC) isoform protein kinase Mζ (PKMζ) might maintain synaptic long-term potentiation (LTP) and long-term memory. However, the role of PKMζ has been challenged by genetic data from PKMζ-knockout mice showing intact LTP and long-term memory. Moreover, the PKMζ inhibitor peptide ζ inhibitory peptide (ZIP) reverses LTP and erases memory in both wild-type and knockout mice. Data from four papers using additional isform-specific genetic approaches have helped to reconcile these conflicting findings. First, a PKMζ-antisense approach showed that LTP and long-term memory in PKMζ-knockout mice are mediated through a compensatory mechanism that depends on another ZIP-sensitive atypical isoform, PKCι/λ. Second, short hairpin RNAs decreasing the amounts of individual atypical isoforms without inducing compensation disrupted memory in different temporal phases. PKCι/λ knockdown disrupted short-term memory, whereas PKMζ knockout specifically erased long-term memory. Third, conditional PKCι/λ knockdown induced compensation by rapidly activating PKMζ to preserve short-term memory. Fourth, a dominant-negative approach in the model system Aplysia revealed that multiple PKCs form PKMs to sustain different types of long-term synaptic facilitation, with atypical PKM maintaining synaptic plasticity similar to LTP. Thus, under physiological conditions, PKMζ is the principal PKC isoform that maintains LTP and long-term memory. PKCι/λ can compensate for PKMζ and because other isoforms could also maintain synaptic facilitation, there may be a hierarchy of compensatory mechanisms maintaining memory if PKMζ malfunctions.

Long-term memories are thought to be stored by persistent modifications in synaptic strength, which alter the connectivity of networks of neurons that underlie cognition and behavior (1, 2). However, most signaling events in neurons last only seconds to minutes. Those that last longer generally alter gene expression in the nucleus or other cell-wide processes (3). Because one neuron can be connected to thousands of other neurons, the signals that affect a neuron as a whole lack the ability to strengthen the synaptic connections between some neurons and not others, which is believed to be essential for encoding specific memories in a network. Thus, identifying the persistent molecular mechanisms that modify transmission at synapses for hours to days and weeks after a brief stimulation is a major challenge for understanding how long-term memories are stored (4).

One approach to identify the signaling mechanisms that sustain enhanced synaptic strength is to study long-term potentiation (LTP)—a persistent, synapse-specific strengthening of excitatory transmission that is triggered by a brief but strong afferent stimulation (5). LTP can be divided into an early phase that exclusively requires post-translational modifications and a late phase that requires de novo protein synthesis (6). These two requirements are also the characteristic features of short-term and long-term memory (3, 7).

The core signaling mechanisms of early-LTP induction by trains of electric stimuli (tetani) are Ca²⁺ influx through N-methyl-d-aspartate-type glutamate receptors (NMDARs) and the consequent stimulation of two main protein kinases, the Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) (5, 8) and protein kinase C (PKC) (9). CaMKII can directly phosphorylate postsynaptic AMPA-type glutamate receptors (AMPARs) to enhance their conductance and also promote trafficking of AMPARs to postsynaptic sites. Activation of CaMKII is limited to ~1 min after strong postsynaptic stimulation (10).

PKC is also activated by tetani, but its response to synaptic stimulation is complex because of the heterogeneity of PKC isoforms. PKC consists of a gene family divided into three classes: conventional PKCs α, β, and γ; novel PKCs δ, ε, η, and θ; and atypical PKCζ and PKCι/λ (human PKCι and rodent PKCs are orthologous genes) (11, 12). Most PKCs consist of a regulatory domain and a catalytic domain. The regulatory domain contains phospholipid and second messenger–binding sites and a pseudosubstrate sequence that helps maintain the catalytic domain in an inactive state. The various classes of PKC are activated by distinct second messengers that bind to the regulatory domain and release this autoinhibition. Conventional PKC isoforms are stimulated by Ca²⁺-triggered binding of anionic phospholipids to their C2 regulatory subdomains and by binding of diacylglycerol (DAG) to their C1 subdomains. Novel PKC isoforms require only DAG for their activation. In contrast, atypical PKC isoforms are activated by various mechanisms, including interaction with lipids such as phosphatidylinositol 3,4,5-trisphosphate (PIP3) or ceramide through their unorthodox C1 regulatory subdomains, binding to other proteins, and phosphorylation of the activation loop of another kinase, phosphoinositide-dependent kinase 1 (PDK1). Thus, the full-length PKCs require a membrane phospholipid environment and second messengers to displace the autoinhibitory pseudosubstrate segments for full activation, and these isoforms translocate from cytosol to membrane during this process. Because the second messengers are rapidly metabolized, the activation of most full-length PKCs in LTP is transient, lasting a few seconds to minutes (13).

PKCι/λ and Early LTP

In contrast to CaMKII and the conventional/novel PKCs, the atypical PKCι/λ has a more persistent action in early LTP. Increases in PKCι/λ phosphorylation on its activation loop, as well as increases in total

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amount of PKCζ/λ, can last 1 hour after tetanization, returning to basal amounts after 3 hours (14, 15). The work of Ren et al. (16) has characterized the mechanism by which PKCζ/λ augments synaptic transmission in early LTP by driving AMPARs to postsynaptic sites to enhance synaptic transmission (Fig. 1A). The peptide inhibitor ζ inhibitory peptide (ZIP) consists of a myristoylated version of the autoinhibitory pseudosubstrate amino acid sequence that is present in the regulatory domain of both PKCζ and PKCζ/λ, and thus, ZIP blocks both PKMζ (protein kinase Mζ) and PKCζ/λ (15, 16). ZIP- and short hairpin RNA (shRNA)-mediated knockdown of PKCζ renders LTP very short-lived (16). Stimulation of phosphatidylinositol 3-kinase (PI3K), which is downstream of CaMKII and mitogen-activated protein kinase, produces PIP3, which leads to activation of PKCζ and increases in postsynaptic AMPAR content and miniature excitatory postsynaptic current (mEPSC) and EPSC magnitude, thus mimicking LTP. All these effects are blocked by ZIP and PKCζ/λ knockdown. PI3K activation also stimulates phosphorylation of Ser818 in the AMPAR GluA1 subunit, an established PKζ site, which contributes to LTP under some conditions (17) but not all (18). In detail, stimulation of PKζ leads to phosphorylation of Ser818 in human embryonic kidney 293 cells, and ectopic expression of GluA1 with an S818A mutation impairs LTP induced by a pairing paradigm (17). It is tempting to speculate that phosphorylation of Ser818 is critical for postsynaptic targeting of homomeric AMPARs, which consist of four GluA1 subunits, rather than that of the more prevalent heterotetrameric GluA1/GluA2 receptors, with GluA1 homomers being required for LTP at certain but not all ages in rodents (19, 20).

On the other hand, PI3K binds to the AMPAR GluA2 subunit (21) and the protein p62, a versatile adaptor protein for various proteins including atypical PKC isoforms, which links PKCζ to AMPARs (22). p62 is also known as sequestosome 1 because of its involvement in autophagy through its binding to polyubiquitinated proteins (23). Ren et al. (16) reported that stimulation of PI3K increases binding of p62 and PKCζ to AMPARs, which is blocked by ZIP. Furthermore, knockdown of p62 impairs LTP. Membrane-permeant peptides mimicking the p62-binding site on GluA1 or the PKCζ/λ-binding site on p62 disrupt the respective interactions and block the increases in postsynaptic AMPAR content and EPSC magnitude by PI3K activation and pairing-induced LTP. In a model (Fig. 1A) for localized increases of postsynaptic AMPAR content mediated by the Ca2+-PI3K-p62/PKCζ/λ pathway, the activity-triggered increase in the p62/PKCζ/λ-AMPAR interaction recruits PKCζ to synapses for extended signaling by PKCζ at these synapses. Because the inhibitors of this pathway suppress potentiation at tetanized synaptic pathways and not non tetanized control pathways, the effects of PKCζ/λ may act at specific synapses, a critical property of LTP and learning. Because p62 binds to the atypical PKC regulatory domain that is not present in PKMζ and full-length PKCζ abundance is very low if not absent in the forebrain (24), PKCζ is the only known candidate that can increase AMPAR activity in this p62-dependent manner.

PKMζ and Late LTP

In contrast to the full-length PKC isoforms that are transiently active in early LTP, the nervous system–specific atypical isoform PKMζ is persistently active in late LTP (25). PKMζ consists of an independent catalytic domain of PKCζ, which is autonomously active because it lacks the autoinhibitory pseudosubstrate in the ζ regulatory domain (13). PKMζ is generated from a dedicated PKMζ mRNA, which contains an open reading frame encoding only the ζ kinase domain that is transcribed by a neural tissue–specific internal promoter within the Prkcz gene (Fig. 1B) (24). The PKMζ mRNA is constitutively transported to the dendrites of neurons (26). However, under basal conditions, the PKMζ mRNA is translationally repressed by multiple micro open reading frames in its long 5′ untranslated region, which prevent ribosomes from reaching and initiating translation of the open reading frame that encodes the kinase (24, 27). However, when LTP is triggered at synapses, Ca2+ influx through the activated NMDAR derepresses the PKMζ mRNA through the concerted action of CaMKII, ERK, PI3K, PKA, and mechanistic target of rapamycin (mTOR) (14). The nascent PKMζ is then fully activated through co-translational phosphorylation by PDK1 (14) and stabilized by signaling mechanisms downstream of brain-derived neurotrophic factor (28) and by binding to the protein KIBRA (29).

The newly synthesized, autonomously active PKMζ is then thought to be “tagged” to specific activated synapses, as shown by synaptic pathway–tagging experiments in hippocampal slices (30) and the translocation of newly synthesized PKMζ to postsynaptic sites during chemically induced LTP of primary cultured neurons (31).

Three lines of evidence indicate that persistent signaling by the increased abundance of PKMζ at synapses then maintains late LTP and long-term memory. First, the persistent increase in steady-state amounts of PKMζ remains stable for hours after LTP induction in hippocampal slices (15, 25) and for days to weeks after learning in vivo, far longer than most other activity-dependent gene products, such as arc or fos (32). The extent of the persistent increase in PKMζ abundance correlates with the degree of synaptic potentiation during LTP maintenance (25) and memory retention during long-term memory storage (32).

Second, the increase of PKMζ at synapses is sufficient to enhance neurotransmission (9), and overexpression of the kinase enhances memory storage (33). Analysis of the enhanced synaptic transmission caused by the postsynaptic perfusion of PKMζ reveals a single mechanism of synaptic potentiation—a doubling of the number of functional postsynaptic AMPARs (34). PKMζ increases postsynaptic receptors by decreasing postsynaptic AMPAR endocytosis mediated by the trafficking protein N-ethylmaleimide–sensitive factor (NSF) through action on the GluA2 subunit of the receptor (35). Further evidence indicates that this mechanism of postsynaptic potentiation maintains both late LTP and long-term memory storage (35–39).

Third, multiple inhibitors of PKMζ reverse LTP maintenance and disrupt established long-term memory. Two pharmacological PKMζ inhibitors, the pseudosubstrate–based peptide ZIP and the small-molecule PKC inhibitor chelerythrine, reverse LTP maintenance when applied many hours after induction and erase long-term memory when applied days to weeks after training (9, 40–43). Overexpression of a dominant-negative mutated version of PKMζ also disrupts established long-term memory (33). In contrast, a broad-spectrum kinase inhibitor, staurosporine, which inhibits conventional and novel PKCs and CaMKII at low doses but not PKMζ blocks the induction but not the maintenance of LTP and long-term memory (9, 40). Furthermore, preventing the removal of AMPARs from postsynaptic sites by inhibiting postsynaptic GluA2 endocytosis blocks the ability of ZIP to reverse LTP maintenance and erase long-term memory (36, 37, 44). These data support the notion that ZIP acts on PKMζ to disrupt the persistence of LTP and memory.

PKMζ-Knockout Mice: Controversy and Compensation

However, genetic evidence from PKMζ-knockout mice cast doubt on the role of PKMζ in LTP and memory (45, 46). First, without PKMζ, the
knockout mice show intact late LTP and long-term memory. Second, the inhibitor ZIP reverses LTP and erases memory in PKMζ-null mice as it does in wild-type mice. These results clearly show that PKMζ is not necessary for LTP or memory and that ZIP has off-target effects that disrupt these processes in the absence of PKMζ. Two hypotheses can explain these results. First, PKMζ is unnecessary for LTP and memory, meaning ZIP acts on targets other than PKMζ. Second, PKMζ is necessary for LTP and long-term memory in wild-type mice, and another molecule, which is also inhibited by ZIP, compensates for PKMζ during LTP and memory in the knockout mice.

To distinguish between these hypotheses, Tsokas et al. (15) used a pharmacogenetic approach. Because the catalytic sites of PKC isoforms are very similar, most PKC inhibitors that compete for substrate binding, such as the pseudosubstrate ZIP, lack isoform specificity. In contrast, the nucleotide sequence of the translation start site in PKMζ mRNA contains sequences found in no other RNA (other than PKCζ mRNA). Tsokas and colleagues identified antisense oligonucleotides complementary to the PKMζ translation site, which blocked the de novo synthesis of PKMζ in wild-type mice without affecting the activity-dependent synthesis of other gene products, including PKCζ/ζ. Tsokas et al. then examined the effect of the PKMζ-antisense on LTP. They confirmed the previous findings of Volk et al. (46) that LTP in wild-type and PKMζ-knockout mice appeared identical. However, the PKMζ-antisense prevented late LTP only in the wild-type mice (15). The PKMζ-antisense had the same differential effect on the two mouse genotypes when tested on spatial long-term memory. Thus, although LTP and spatial memory in the wild-type and PKMζ-knockout mice appear similar, their underlying molecular mechanisms are different.

To characterize the mechanism for the compensation in the PKMζ-null mice, Tsokas et al. analyzed the complete PKC isoform family in the hippocampus. The results revealed basal increases in two isoforms in the knockout mice—PKCζ and the atypical isoform PKCζ/ζ. Both isoforms are activated in the early phase of LTP in wild-type animals, with PKCζ activation lasting for seconds after synaptic stimulation (13) and
PKC/ι activation lasting about 1 hour (14). Because PKC/ι is the most closely related PKC isom in PKMζ, it was a likely candidate to compensate for PKMζ in the knockout mice. To examine this possibility, Tsokas et al. first compared the activation of PKC/ι during LTP maintenance in wild-type and PKMζ-null mice. During late LTP in wild-type mice, the increases in PKC/ι activation, as measured by increased PKC/ι phosphorylation by PDK1 and increased total amounts of PKC/ι, returned to baseline after 3 hours. In contrast, during late LTP in PKMζ-null mice, the increases in phosphorylated and total PKC/ι were maintained for at least 3 hours (Fig. 1C).

The double dissociation revealed from experiments with the PKMζ-antisense and PKC/ι inhibitor is consistent with the hypothesis that PKMζ is important for LTP and long-term memory in wild-type mice, and that compensatory mechanisms maintain these processes in PKMζ-null mice. The mechanism compensating for the loss of PKMζ is to prolong the activation of PKC/ι, which is important for early LTP in wild-type animals (16), into late LTP in the PKMζ-null mice (Fig. 1).

Specific Functions of PKC/ι and PKMζ in LTP and Memory

In a second study, Wang et al. (47) distinguished between the roles of PKC/ι and PKMζ in synaptic plasticity and memory by using shRNA to decrease the abundance of the isoforms individually. The viral expression of shRNA in the hippocampus reduced the amount of each isoform by ~60 to 75% without inducing a compensatory change in the other. When the amount of PKC/ι was decreased before stimulation, early LTP and short-term memory were blocked, consistent with the studies of Ren et al. (16). Knockdown before stimulation also prevented the late phase of LTP and long-term memory. In contrast, when the amount of PKMζ was decreased, early LTP and short-term memory were spared, and the late phase of LTP and long-term memory was specifically eliminated.

Because the PKC/ι knockdown affected short-term memory, it could have either prevented the induction of long-term memory or, if PKC/ι acted persistently in memory like PKMζ, disrupted the maintenance of long-term memory. To distinguish between these possibilities, Wang et al. injected the viruses expressing PKC/ι and PKMζ shRNA several days after training and examined memory retention 1 month later. The results revealed that only the knockdown of PKMζ and not PKC/ι suppressed the storage of previously established long-term memory.

Compensation for PKC/ι by PKMζ in Mice with Conditional Knockout of PKC/ι

Because global PKC/ι-null mice are embryonically lethal (48), Sheng et al. (49) further examined PKC/ι function in conditional knockout mice. They injected an adeno-associated virus (AAV)–expressing Cre recombinase in the hippocampus of PKC/ι-floxed mice to reduce the amount of the isoform by 85 to 90%. In contrast to pharmacological blockade or shRNA knockdown of PKC/ι, PKC/ι conditional knockout mice showed early LTP and short-term memory. No changes in the basal abundance of PKMζ were detected. However, on analysis of early LTP and short-term memory formation, Sheng et al. observed that PKMζ was formed more rapidly in PKC/ι conditional knockout mice than in wild-type mice, suggesting possible compensation (Fig. 1D).

To examine the function of PKMζ in the PKC/ι conditional knockout mice, Sheng and colleagues took advantage of previous findings that PKC/ι and PKMζ enhance postsynaptic AMPAR-mediated transmission by different mechanisms of potentiation—PKC/ι by enhancing exocytosis through action on the GluA1 subunit (16) and PKMζ by blocking endocytosis through NSF–GluA2 subunit interactions (35). Using an inhibitor of exocytosis and a peptide that blocks NSF–GluA2 interactions, Sheng et al. observed a double dissociation—early LTP in the wild-type mouse but not PKC/ι conditional knockout mice required exocytosis, whereas early LTP in the PKC/ι conditional knockout mice but not wild-type mice required NSF–GluA2 interactions. Thus, a compensatory switch in early LTP occurs in the PKC/ι conditional knockout mice from the synaptic potentiation mechanism of PKC/ι to that of PKMζ (Fig. 1D).

Suboptimal Learning and Memory Produced by Compensation Between Atypical PKCs

The knockout mouse studies revealed that each atypical PKC can compensate for the function of the other to preserve short- and long-term memory. However, if each isoform can mediate both phases of memory, then why are the roles of the two isoforms normally differentiated—with PKC/ι restricted to short-term memory and PKMζ to long-term memory? Tsokas et al. and Sheng et al. addressed this question by asking whether learning and memory in knockout mice with only a single atypical PKC might be worse than wild-type mice with both. To reveal possible deficits in behavior, Tsokas and colleagues (15) made memory formation progressively harder to acquire. First, on examining the ability of the mice to avoid a shock zone on a rotating platform, they decreased the duration of the training periods so that the acquisition of spatial memory accumulated slowly over several days rather than rapidly in a single day. The wild-type mice learned to avoid the shock zone by exploring the whole apparatus to find the safest location furthest from the shock zone. In contrast, the PKMζ-knockout mice avoided the shock by remaining in a region close to it, rarely moving from that location to explore the full apparatus, a relatively inefficient strategy that results in more shocks. Next, Tsokas et al. tested PKMζ-knockout and wild-type mice on a battery of learning and memory tasks that involves placing objects in novel locations and contexts. Whereas the PKMζ-knockout mice and wild-type mice performed the simple tasks equally well, the PKMζ-knockout mice could not learn or remember a complex version of the task involving multiple object-locations unlike the wild-type mice. In similar experiments on PKC/ι conditional knockout mice, Sheng et al. made hippocampus-dependent versions of fear conditioning and spatial tasks more difficult by reducing the number of training trials (49). The PKC/ι conditional knockout mice performed worse than wild-type mice in the more difficult versions of both tasks.

Thus, mice with two atypical PKCs perform better in learning and memory tasks than those with only one. In cognitively complex tasks, multiple short-term memories might have to be integrated to determine the optimal strategy to be stored in long-term memory. Therefore, in the PKMζ-knockout mice, keeping short- and long-term memories separate might be problematic if only PKC/ι is available for both short- and
long-term memory storage within neurons. Likewise, in the PKCz/λ conditional knockout mice, rapid PKMζ activation may not be as efficient or sensitive to stimulation as PKCζ/λ, such that only a few short-term experiences might not be sufficient to encode a long-term memory. Thus, the advantages of separate, specialized atypical PKCs for storing short-term and long-term memories may provide selection pressure that maintains both atypical isoforms in vertebrate evolution.

A Plethora of PKMs

The PKMζ-knockout mice show that the other atypical isoform, PKCζ/λ, can compensate for the loss of PKMζ by becoming persistently active to maintain late LTP and long-term memory. However, could persistent action of the conventional and novel PKCs also maintain long-term memory? Work in the invertebrate model system of learning and memory Aplysia californica suggests that they may (50). The mollusk A. californica produces a single isoform for each of the three PKC classes in neural tissue (50). The Aplysia-atypical PKC also forms a PKM by proteolytic cleavage of the full-length atypical PKC by calpain rather than through translation of a PKMζ mRNA, a mechanism of PKM formation found only for PKMζ in vertebrates (51, 52). In Aplysia, all three PKCs can form PKMs through cleavage by various forms of calpain (50).

Glanzman and colleagues showed that ZIP and chelerythrine erase behavioral long-term sensitization of siphon-withdrawal reflexes when the inhibitors are introduced into the nervous system of the animals 1 week after training (33). Both agents also reverse the maintenance of long-term facilitation of AMPAR-type glutamatergic synaptic transmission between cultured identified neurons, a form of synaptic plasticity that underlies the behavioral sensitization (54, 55).

Hu et al. (50) have identified the PKC isoforms that maintain the persistent synaptic enhancement. They examined two forms of long-term synaptic facilitation: the nonassociative form of plasticity studied by Glanzman, which is induced by the application of the neuromodulator serotonin, and an activity-dependent form induced by the combination of serotonin and synapse activation, which is thought to underlie classical conditioning and is similar to LTP. One day after the stimulation that produced the forms of long-term plasticity, they overexpressed dominant-negative versions of each PKM isoform either pre-or post-synaptically so as to localize the site of the persistent action of the PKM isoform in long-term maintenance.

In a remarkable example of conservation of function across phyla, blocking of the postsynaptic action of Aplysia-atypical PKM, which is the isoform most similar to vertebrate PKMζ, reversed the maintenance of the LTP-like, activity-dependent long-term facilitation (50). The dominant-negative atypical PKM also reversed nonassociative long-term facilitation when injected presynaptically. However, expression of a dominant-negative novel PKM also reversed the maintenance of both forms of long-term facilitation when overexpressed in the same sites. Conversely, expression of dominant-negative conventional PKM reversed maintenance when overexpressed in the complementary neurons—presynaptically in associative long-term facilitation and post-synaptically in nonassociative long-term facilitation. The reversal of specific forms of long-term synaptic facilitation by specific dominant-negative PKMs implied that PKC isoforms in Aplysia do not compensate for each other after the relatively rapid overexpression of the dominant negative PKMs. A second study by the same group found that atypical PKM and conventional PKM maintained associative and nonassociative forms of long-term plasticity, respectively, at distinct synapses even within the same postsynaptic neuron (56). Thus, the work in Aplysia suggests that multiple PKC isoforms, in addition to atypical PKC, can maintain long-term synaptic enhancement by forming persistently active PKMs.

Conclusions

These four studies using various genetic methods to examine PKC isoforms provide fundamental insights into the molecular mechanisms of memory maintenance and help resolve the controversy over PKMζ in long-term memory storage, in which the pharmacological and dominant-negative data appeared to conflict with the knockout mouse data. The results of the pharmacogenetic analysis of the PKMζ-knockout and the shRNA-mediated knockdown of PKMζ demonstrated that PKMζ is the principal PKC isoform maintaining LTP and long-term memory storage under physiological conditions in wild-type mice (15, 47). However, these findings, together with those from the PKCζ/λ conditional knockout mice (49), also revealed that the heterogeneity of PKC isoforms provides flexibility to the molecular mechanisms that sustain the fundamental processes of LTP and memory. In the PKMζ-knockout mice, another atypical PKC that is also blocked by the inhibitors used to probe PKMζ function, ZIP and chelerythrine, compensated for PKMζ by extending its role in early LTP and short-term memory into late LTP and long-term memory (15). The converse also occurred: In the PKCζ/λ conditional knockout mice, PKMζ was activated more rapidly to compensate for the loss of PKCζ/λ in early LTP and short-term memory (49). Compensation in memory function may not be restricted to the atypical PKCs. In the PKMζ-knockout mice, both PKCζ/λ and the conventional PKCζ/λ increased in hippocampus (15). Moreover, studies of a model system with fewer PKC isoforms demonstrated that not only atypical PKC but also conventional and novel PKCs could form PKMs to sustain the long-term synaptic facilitation that underlies memory maintenance (50). The generality of PKM formation from any PKC isozone raises the possibility that other forms of synaptic plasticity and memory not yet characterized could be sustained by the persistent action of different PKCs.

The common feature linking all the physiological and compensatory PKC isoform–specific mechanisms is that the persistent action of the isoform sustains the maintenance of the memory. Thus, the genetic studies raise the fundamental question of the nature of the mechanisms by which persistent increases in autonomously active PKMζ can be sustained for weeks (32). Is the persistence due to increased PKMζ synthesis or decreased degradation? How might these mechanisms result in persistent increases in the kinase that remain compartmentalized to specific synapses? How many synapses contain PKMζ to store a memory? Are there mechanisms to keep its distribution sparse, so as not to “saturate” the synapses of a neuron with PKMζ as more memories are stored? Do PKCζ/λ or PKMs of other isozymes use isoform-specific mechanisms for maintenance, or do they engage a common set of maintenance mechanisms when PKMζ is no longer available? Which proteins are the relevant targets for phosphorylation by these kinases? Epigenetic changes are also implicated in long-term memory maintenance (57, 58). PKMζ may act both upstream and downstream in these epigenetic mechanisms. Phosphorylation by nuclear PKMζ maintains histone acetylation during memory storage (59), and decreased DNA methylation of the Prkcz gene after memory formation leads to increase amounts of PKMζ mRNA (60). How might the epigenetic regulation of PKMζ and the nuclear effects of PKMζ interact with its role in synaptic potentiation? Addressing these questions in future studies of PKMζ and compensating PKCs will lead to an even deeper understanding of how long-term memories are stored.
REFERENCES AND NOTES


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