Dendritic Transport and Localization of Protein Kinase Mζ mRNA: IMPLICATIONS FOR MOLECULAR MEMORY CONSOLIDATION*

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Abstract

Protein kinase Mζ (PKMζ) is an atypical protein kinase C isoform that has been implicated in the protein synthesis-dependent maintenance of long term potentiation and memory storage in the brain. Synapse-associated kinases are uniquely positioned to promote enduring consolidation of structural and functional modifications at the synapse, provided that kinase mRNA is available on site for local input-specific translation. We now report that the mRNA encoding PKMζ is rapidly transported and specifically localized to synaptodendritic neuronal domains. Transport of PKMζ mRNA is specified by two cis-acting dendritic targeting elements (Mζ DTEs). Mζ DTE1, located at the interface of the 5′-untranslated region and the open reading frame, directs somato-dendritic export of the mRNA. Mζ DTE2, in contrast, is located in the 3′-untranslated region and is required for delivery of the mRNA to distal dendritic segments. Colocalization with translational repressor BC1 RNA in hippocampal dendrites suggests that PKMζ mRNA may be subject to translational control in local domains. Dendritic localization of PKMζ mRNA provides a molecular basis for the functional integration of synaptic signal transduction and translational control pathways.

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Synapses, the local sites of communication between neurons, are elemental modules of information processing in the nervous system. Subject to activity- and experience-dependent modification, synaptic connections are also sites of plastic modulation of interneuronal communication. It is thought that various forms of such modulation, collectively called synaptic plasticity, are determinants of information storage at the synapse that underlie neural development and higher brain functions, including learning and memory. Numerous mechanisms, not mutually exclusive, have been proposed to serve as molecular substrata of plastic events at the synapse, prominent among them phosphorylation signaling pathways and protein synthetic pathways.
De novo synthesis of proteins is required for the long term modulation of synaptic strength. Characterized by highly mosaic and dynamic protein repertoires, synaptodendritic microdomains have in recent years been recognized to generate and maintain at least part of such dynamic diversity by local synthesis on site (for reviews, see Refs. 1-6). Prerequisite for such a mechanism is the targeted transport of relevant mRNAs to subsynaptic sites of translational competence.

Long lasting synaptic plasticity is thus likely to require dendritic RNA transport, localization, and translation. In contrast, post-translational modification of existing synaptic proteins is presumably responsible for initial changes in synaptic strength. Diverse phosphorylation signaling pathways have been suggested to contribute functionally to the plastic modification of synaptic connections. Specifically, various types of protein kinases have been implicated in long term potentiation (LTP), an experimental model of synaptic plasticity and memory formation. Such kinases include members of the calcium/calmodulin-dependent protein kinase (CaMK) family, the cAMP-dependent protein kinase family, the mitogen-activated protein kinase family, and the protein kinase C (PKC) family (reviewed in Ref. 7).

Recently, the autonomously active isoform of atypical protein kinase Cζ, called protein kinase Mζ (PKMζ), has been reported to be required for the maintenance of LTP (8). It was further shown that induction of a mouse PKMζ transgene in Drosophila enhanced memory in olfactory learning paradigms (9), suggesting a key role for PKMζ-mediated signal transduction in the maintenance of synaptic plasticity and behavioral memory (10). PKMζ is autonomously active because it is the independent catalytic domain of PKCζ and is not autoinhibited by the pseudosubstrate of the regulatory domain of PKCζ. However, PKMζ is not a proteolytic fragment of PKCζ as we originally hypothesized (11), but is instead synthesized directly from a brain-specific PKMζ mRNA (12). Formation of PKMζ during LTP maintenance has been shown to require increased de novo protein synthesis (12,13). The combined observations therefore prompt a scenario in which PKMζ mRNA is locally available at synapses for site-specific translation. However, such local availability and its fundamental prerequisite, the targeted delivery of PKMζ mRNA to synaptodendritic sites, has hitherto not been documented.

Here we present a functional dissection of the dendritic targeting competence of PKMζ mRNA. We report that PKMζ mRNA is specifically delivered to distal dendritic domains. We demonstrate that such transport is specified by two dendritic targeting elements that are contained within the RNA. In hippocampal dendrites, PKMζ mRNA was found colocalized with the translational modulator BC1 RNA. We propose that dendritic delivery of PKMζ mRNA to translationally competent synaptic sites enables the phosphorylation signaling pathway and the local protein synthetic pathway to synergize in the implementation of long term synaptic plasticity.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**

Primary cultures of hippocampal neurons were prepared as described by Goslin et al. (14). The cells were dissociated from embryonic day 18 rat hippocampi and were plated onto polylysine-treated glass coverslips. Primary cultures of sympathetic neurons were generated as described (15,16). Superior cervical ganglia from embryonic day 20–21 Sprague-Dawley rat embryos were dissociated, and the cells were plated on polylysine-treated glass coverslips. Basement membrane extract (Matrigel; Collaborative Biomedical Products, Bedford, MA) was used at
100 μg/ml to induce dendritic growth. The neurons were fixed in 4% formaldehyde (made from paraformaldehyde) and 4% sucrose in 140 mOs NaCl, 15 mOs phosphate buffer, pH 7.3, at room temperature for 20 min. The coverslips with fixed cells were stored in 70% ethanol at −20 °C until further processing (17).

**RNA Preparation**

The following transcripts were generated for microinjection experiments: (1) PKMζ mRNA Segment 1 (1935 nt) from plasmid pPKMζ(48–1982) (12), representing PKMζ mRNA nt 48–1982, inserted between HindIII and XbaI in pBlueScript SK(−) (Stratagene, La Jolla, CA), linearized with XbaI; (2) PKMζ mRNA Segment 2 (1851 nt) from plasmid pPKMζ(48–1982), linearized with NsiI; (3) PKMζ mRNA Segment 3 (859 nt) from plasmid pPKMζ(48–1982), linearized with SacI; and (4) PKMζ mRNA Segment 4 (300 nt) from plasmid pPKMζ(48–1982), linearized with PsI. Analogously, PKMζ mRNA Segment 5 (1635 nt) represents PKMζ mRNA nt 348–1982, and PKMζ mRNA Segment 6 (1136 nt) represents PKMζ mRNA nt 847–1982. Segments 5 and 6 were transcribed from pPKMζΔ1–347 and pPKMζΔ1–846, respectively. These plasmids, derived from pPKMζ(48–1982), were linearized with XbaI. Finally, pPKMζΔ1905–1946 (linearized with XbaI) was used to generate PKMζΔ1905–1946 mRNA (1893 nt), containing a 42-nt deletion mutation. This mRNA is in the following referred to as PKMζ mRNA Segment Δ.

Other RNAs, injected for reference, included full-length BC1 RNA (used as a reference RNA that is transported along the entire dendritic length). They further included 3′ BC1 RNA, U4 RNA, and irrelevant polylinker-derived RNAs, all of which were used as reference RNAs that remain restricted to the soma. Intracellular localization of these reference RNAs, and the respective transcription vectors to generate them, have been described by Muslimov et al. (16). All RNAs were prepared from linearized plasmids, using SP6, T3, or T7 RNA polymerase, according to the manufacturer's protocols (Promega, Madison, WI). Following transcription in the presence of 35S-UTP, excess unlabeled UTP was added to the reaction mixture to ensure that labeled transcripts were full-length. The integrity of all transcripts was monitored as described (16).

For **in situ** hybridization, probes were prepared as follows. A probe specific for PKMζ mRNA was generated from plasmid pPKM180, corresponding to 180 nt of the 5′-most region of PKMζ mRNA (18), cloned into pBluescript KS(+) (Stratagene). This probe does not recognize PKCζ mRNA or other atypical PKC/M mRNAs (12). For **in vitro** transcription, the plasmid was linearized with ApaI for “antisense” and with SacI for “sense” strand probe transcription. BC1 RNA probes were generated from plasmid pMK1 (19). Neuron-specific enolase (NSE) mRNA probes were generated from plasmid pNSEISH. A full-length NSE cDNA clone, obtained from Dr. Forss-Petter (20), was digested with the BglII to generate a 1.2-kb fragment containing most of the ORF. This fragment was blunt ended and ligated into pBluescript KSII (+) to yield pNSEISH. The plasmid was linearized with XhoI for **in vitro** transcription with T7 RNA polymerase to generate sense strand probes and with Smal for **in vitro** transcription with T3 RNA polymerase to generate antisense strand probes.

**In Situ Hybridization**

Male Sprague-Dawley rats (~250 g) were used. The animals were perfusion-fixed with 4% formaldehyde (freshly prepared from paraformaldehyde) in phosphate-buffered saline, the brains were sectioned at 10 μm, and the specimens were post-fixed by UV illumination (21). Prehybridization, hybridization, and subsequent processing steps were performed with brain sections and cultured neurons as described previously (17,19,21). High stringency washes were performed at 50 °C (hybridization to BC1 RNA) or 45 °C (hybridization to PKMζ and NSE mRNAs).
RNA Microinjection

RNA microinjection was performed as described in Muslimov et al. (16). Fine tipped microinjection needles were used to pressure inject neurons with RNA. Injected RNAs were \(^{35}\text{S}\)-radiolabeled at \(3 \times 10^6\) cpm/μl. RNA was microinjected at volumes of several femtoliters/pulse. Lucifer yellow (0.4%) was coinjected for calibration purposes. The neurons were incubated for 4 h at 35 °C (unless stated otherwise) prior to fixation and emulsion autoradiography. For PKM\(\zeta\) mRNA, the number of dendrites analyzed was as follows: Segment 1, 18 dendrites; Segment 2, 12 dendrites; Segment 3, 24 dendrites; Segment 4, 15 dendrites; Segment 5, 15 dendrites; Segment 6, 16 dendrites; and Segment \(\Delta\), 15 dendrites.

Data Evaluation

Emulsion autoradiography was performed as described (16,21). The specimens were analyzed and photographed on a Nikon Microphot-FXA microscope using dark field and phase contrast optics. Digital images were acquired using a Sony 3CCD DKC-5000 camera or a Photometrics CoolSnapHQ camera.

Dendritic distribution profiles were established by measuring silver grain densities along dendrites. A dendritic labeling signal was considered significant if it exceeded background levels by a factor of at least three (16). Background was determined in areas of equal size in “sense” strand controls. Dendrites were identified by morphology as described (22). To analyze transport competence of microinjected RNAs, silver grain densities were established along dendritic extents of sympathetic neurons in 40-μm intervals. MetaMorph software (Universal Imaging Corporation, Downingtown, PA) and KaleidaGraph software (Synergy Software, Reading, PA) were used for quantitative analysis. NIH BLAST software was used for sequence comparisons (23), and Mfold software was used for RNA secondary structure predictions (24).

RESULTS

PKM\(\zeta\) mRNA Is Localized to Somatodendritic Domains in Cultured Neurons

PKM\(\zeta\) is translated in brain from a unique mRNA that encodes the catalytic but not the regulatory domain of PKC\(\zeta\) (12). This PKM\(\zeta\)-encoding mRNA is transcribed from an alternative internal promoter within a single PKC\(\zeta\) gene (12,25). To establish whether PKM\(\zeta\) mRNA is localized to dendrites, we first performed in situ hybridization with hippocampal neurons in primary culture, using a PKM\(\zeta\)-specific probe (see “Experimental Procedures”). Fig. 1 shows that substantial signal intensities, indicating the presence of PKM\(\zeta\) mRNA, were observed in both somata and dendrites of hippocampal neurons. No significant signal was detectable along axonal processes. In dendrites, the signal was typically of heterogeneous and clustered appearance, the latter often evidenced at branch points or at points of intersection with other processes. Such localization may be interpreted to indicate that PKM\(\zeta\) mRNA is enriched at synaptic sites.

Analogous results were obtained with sympathetic neurons in culture (Fig. 2). Again, the labeling signal for PKM\(\zeta\) mRNA was discontinuously distributed along dendritic arborizations. Similar to hippocampal neurons in culture, PKM\(\zeta\) mRNA signal in dendrites was observed along the full dendritic length, including the distal-most dendritic segments. Axonal processes were devoid of specific labeling. On average, signal intensities for PKM\(\zeta\) mRNA were about 10 times lower in sympathetic neurons in culture than in hippocampal neurons in culture (a fact that was compensated for in Fig. 2 by correspondingly longer autoradiographic exposure times). In summary, the results show 1) that PKM\(\zeta\) mRNA is present in significant amounts in somatodendritic domains in neurons, 2) that it is localized in clustered fashion throughout
proximal and distal segments of dendritic arborizations, and 3) that somatodendritic expression levels are cell type-dependent.

In Vivo, PKMζ mRNA Codistributes with BC1 RNA in Dendritic Layers

We next sought to ascertain dendritic localization of PKMζ mRNA *in vivo*. PKMζ mRNA was found heterogeneously distributed in adult rat brain (Fig. 3). Neocortex, hippocampus, striatum, and thalamic nuclei were among the brain areas that displayed the most robust PKMζ mRNA expression levels. To establish degrees of dendritic localization, we compared the expression pattern of PKMζ mRNA in hippocampus with that of BC1 RNA, a nontranslatable *bona fide* dendritic RNA (19,26) that has recently been shown to operate as a repressor of translation initiation (27). In addition, NSE mRNA was chosen as a representative of somatically restricted neuronal RNAs (28).

PKMζ mRNA and BC1 RNA were identified at substantial levels not only in stratum pyramidale but also in stratum radiatum and stratum lacunosum moleculare of Ammon’s horn, layers that contain medial and distal apical dendritic arborizations, respectively, of hippocampal pyramidal cells (Fig. 3, A and B). Expression of both RNAs took the characteristic form of a gradient with intensity levels diminishing in the CA3 to CA1 direction. Furthermore, although both PKMζ mRNA and BC1 RNA were strongly expressed in the hilar region, only relatively low amounts of either RNA were detected in somatic or dendritic layers of the dentate gyrus. These results indicate coexpression as well as codistribution of PKMζ mRNA and BC1 RNA in the hippocampal formation.

Do PKMζ mRNA and BC1 RNA also colocalize along dendrites of hippocampal pyramidal cells? To address this question, we analyzed expression levels of PKMζ mRNA in the CA3 field in comparison with BC1 RNA, NSE mRNA, and the mRNA encoding microtubule-associated protein 2 (MAP2) (29-31) (Fig. 4). Although PKMζ mRNA and BC1 RNA were strongly expressed in medial and distal dendritic layers, lower levels of both RNAs were detected in the stratum lucidum, the layer through which the proximal segments of pyramidal apical dendrites traverse. This evaluation was confirmed by quantitative analysis along the pyramidale to lacunosum moleculare extent (Fig. 4E). Of note, proximal segments of CA3 apical pyramidal cell dendrites, innervated by mossy fibers in stratum lucidum, have been shown to display both a lower density of N-methyl-D-aspartate receptor activity and less regulation of AMPA receptors during LTP than distal segments of these dendrites (32,33).

The dendritic expression profile of PKMζ mRNA and BC1 RNA in CA3 is distinctive as it is distinguished from patterns of other dendritic RNAs. For example, we detected MAP2 mRNA at approximately equal levels in both stratum pyramidale and stratum lucidum (Fig. 4, C and E). At the same time, the highest relative levels of MAP2 mRNA were seen in dendritic stratum radiatum but not extending into stratum lacunosum moleculare, the distal-most dendritic layer (Fig. 4, C and E; see also Refs. 29 and 34). The PKMζ/BC1 profile is also not shared with dendritic CaMKIIα mRNA because the latter is expressed at highest relative levels in stratum pyramidale and at uniform but overall lower relative levels in strata lucidum, radiatum, and lacunosum moleculare (35,36).

Taken together, the results show that PKMζ mRNA and BC1 RNA are targeted to colocalize in a characteristic pattern along CA3 pyramidal cell apical dendrites. Such colocalization is indicative of functional interactions between PKMζ mRNA and BC1 RNA, a translational modulator, in the regulation of PKMζ synthesis in pyramidal cell dendrites. This hypothesis remains open to future investigation.
Dendritic Transport of PKMζ mRNA Is Specified by Two Cis-acting Targeting Elements

The above data show that PKMζ mRNA is localized to dendritic domains of neurons both in culture and in vivo. Is the RNA actively transported to dendrites, and if so, what is/are the code(s) that specify such transport? To address these questions, we used a microinjection paradigm with in vitro synthesized RNAs (16,37). Radiolabeled RNAs, including PKMζ mRNA segments of desired lengths and sequence contents, were introduced into cultured sympathetic neurons by somatic microinjection. After appropriate post-injection time intervals to allow for transport, the cells were fixed, and dendritic delivery was ascertained by emulsion autoradiography.

Cis-acting DTEs, inasmuch as they have been identified in localized neuronal mRNAs, often reside in 3′-UTRs. We therefore decided to examine dendritic targeting competence by working initially in the 3′ to 5′ direction. A series of PKMζ mRNA segments was generated by successive trimming in this direction to pinpoint presumed DTEs within the PKMζ mRNA sequence (Fig. 5). As shown in Fig. 6, a segment spanning nucleotides 48–1982 (Segment 1) was transported along dendrites to localize in a fashion indistinguishable from endogenous PKMζ mRNA (Figs. 1 and 2) or from dendritic BC1 RNA (16). The results indicate that this segment is delivered along the entire dendritic extent to reach the dendritic tips. Time course experiments were performed as described previously (16) and showed that dendritic transport was rapid at 460 ± 70 μm/h (data not shown). Axonal transport was not observed in these or any of the following experiments.

In clear contrast to Segment 1, a PKMζ mRNA segment comprising nt 48–1898 (Segment 2) produced a much more restricted dendritic localization pattern. In this case, the signal distribution indicates that the RNA, although clearly delivered to dendrites, failed to reach distal dendritic segments (Fig. 7). The combined results thus suggest that a cis-acting element that is necessary for distal dendritic targeting is contained within section 1899–1982 of PKMζ mRNA. Further 3′ to 5′ length reduction to nt 906 (Segment 3) did not result in any additional decrease in the proximo-distal extent of dendritic localization (Fig. 7), a result suggesting that section 907–1898 of PKMζ mRNA does not contain cis-acting elements of significant dendritic targeting competence. However, when PKMζ mRNA was even further trimmed to contain only nt 48–347 (Segment 4), we found that the RNA now failed to exit the soma altogether (Fig. 7). We conclude that section 348–906 of PKMζ mRNA contains a cis-acting targeting element that is necessary to export the RNA from the soma to dendrites.

For an independent corroboration of these data, we generated additional deletion constructs. Because a DTE at the 5′-UTR/ORF interface was unexpected, we re-examined dendritic targeting competence by successive trimming in the reverse, i.e. the 5′ to 3′ direction. PKMζ mRNA Segment 5, lacking nt 1–347, was transported to dendrites in a manner that was not significantly different from Segment 1 (Fig. 8). However, Segment 6, lacking nt 1–846, did not enter dendrites to any noticeable degree (Fig. 8). These results confirm the above data, and taken together, they indicate that a cis-acting DTE is contained in a segment spanning nt 348–846 of PKMζ mRNA.

As described above, a second cis-acting DTE is contained within a 84-nt segment (nt 1899–1982) of PKMζ mRNA. Secondary structure analysis (Mfold) of this segment predicted a stable 44-nt stem-loop spanning nt 1905–1948 (see also Fig. 10 and “Discussion”). To probe the relevance of the 44-nt segment for the dendritic targeting competence of PKMζ mRNA, we used construct pPKMζΔ1905–1946 to generate a mutant PKMζ mRNA, called Segment Δ, that was lacking 42 of the 44 nt in the predicted stem-loop. Following microinjection, we observed that Segment Δ was delivered to dendrites but failed to reach distal dendritic arborizations and tips (Fig. 8). Segment Δ thus produced a dendritic signal that was similar in extent to the one produced by Segment 2 (see above). The combined results indicate that a second DTE is
contained within a 42-nt segment of the PKMζ 3′-UTR, a segment that is predicted to assume a stable stem-loop conformation.

Fig. 9 provides a quantitative synopsis of the above described transport experiments. Our data suggest that two DTEs are needed to specify targeting of PKMζ mRNA to dendritic destinations. The first element, straddling the interface of the 5′-UTR with the ORF, is necessary for somato-dendritic export but is not sufficient to direct the RNA to distal domains in dendrites. This element is henceforth called Mζ DTE1. The second element, a stem-loop structure residing in the 3′-UTR and henceforth called Mζ DTE2, is required for distal dendritic targeting.

DISCUSSION

A basic requirement for the long term modulation of synaptic efficacy is the need for de novo synthesis of proteins. Increasing evidence suggests that at least part of this requirement is met by local translation of synaptodendritic mRNAs (reviewed in Refs. 1-6). This mechanism requires the presence of a select group of mRNAs at the synapse where they would be subject to local translational control (discussed in Refs. 3, 5, and 27). The presence in dendrites of mRNAs encoding autonomously active kinases is of particular relevance because it would provide a molecular basis, by functional integration of local signal transduction pathways and translational control mechanisms, for the consolidation of long term information storage at the synapse.

PKMζ mRNA: Dendritic Targeting Elements

We report that the mRNA encoding PKMζ is specifically transported to dendrites. At about 460 μm/h, the transport rate is comparable with those of other dendritic RNAs (e.g. dendritic BC1 RNA is transported at 490 μm/h) (see Ref. 16 and reviews quoted above.) To our initial surprise, we found that two DTEs are required for PKMζ mRNA transport to distal dendritic domains. One of them (Mζ DTE1) resides in a 499-nt segment that is spanning parts of both 5′-UTR and ORF, whereas the second one (Mζ DTE2) resides in a 42-nt segment of the 3′-UTR. Although Mζ DTE1 contains a code to specify export of the mRNA from soma to dendrites, Mζ DTE2 is required for effective targeting to distal dendritic domains. The location of a cis-acting targeting element in the 5′-UTR and/or ORF is unusual but not unprecedented. Although such elements frequently reside in 3′-UTRs (5,38), the mRNA encoding rat vasopressin contains a DTE that is located in the ORF, possibly extending into the 3′-UTR (39). Drosophila gurken mRNA contains several cis-acting elements, specifying RNA localization during different developmental stages, that are contained in the 5′-UTR, the ORF, and the 3′-UTR (40). Thus, although a position in 3′-UTRs appears to be common for many cis-acting localization or transport elements, our results and previous data are in agreement that such location may not be universally required, or even relevant, for effective targeting.

The bipartite structure of PKMζ mRNA targeting competence invites comparison with other transported and/or localized RNAs. A sequence of 12 nt (nt 781–792) from the Mζ DTE1 containing segment of PKMζ mRNA (nt 348–906) matches (11 nt of 12) part of the targeting-competent 5′ domain of dendritic BC1 RNA (16). Although the significance of such sequence motifs for dendritic transport remains to be established, data obtained with BC1 RNA indicate that secondary structure context is a major determinant of transport competence.2 No other sequence similarities with the Mζ DTE1-containing segment of PKMζ mRNA were revealed by data base searches. The 42-nt segment containing Mζ DTE2 was not found to exhibit any obvious sequence similarity with dendritically or otherwise localized RNAs that have been

2I. A. Muslimov and H. Tiedge, unpublished observation.
reported to date. However, it is predicted to form a stable stem-loop structure with a prominent asymmetric A/G bulge (Fig. 10). Noncanonical purine-purine base pairing in such bulges is a structural feature of direct relevance for RNA-protein interactions (41,42). It should be noted, however, that the predicted stem-loop structure will remain hypothetical until corroborated by techniques such as enzymatic and chemical probing (43).

Bipartite dendritic targeting determinants may be indicative of underlying mechanistic requirements. In an axonal RNA transport model system in vivo, it has recently been shown that BC1 RNA is delivered to local sites of protein synthetic capacity in a two-step process that is functionally dependent on the sequential participation of microtubules and actin filaments (44). It is plausible that two cis-acting elements are required to cooperate in modular fashion if delivery to, and anchoring at, local target sites necessitate cytoskeletal switching. We do not know at this time whether dendritic transport and localization of PKMζ mRNA also make sequential use of different cytoskeletal systems. Nonetheless, it appears reasonable to assume that PKMζ mRNA lacking MζDTE2 fails to establish stable association with local docking elements at distal dendritic sites. Such “localization failure” may consequently lead to release, diffusion, and ultimate degradation of the RNA.

It is significant that PKMζ mRNA is targeted to the distal-most dendritic segments, a feature that is shared by only few other known dendritic RNAs, among them BC1 RNA and CaMKIIα mRNA (19,35). (An issue of current debate, dendritic transport of CaMKIIα mRNA may also be controlled by more than one targeting element; discussed in Refs. 36,38,45, and 46). Such distal dendritic location indicates that the mRNA is required and available for local translation throughout dendrites, in contrast to such dendritic mRNAs that are restricted to proximal or proximo-medial dendritic segments (e.g. GAP-43 mRNA, MAP2 mRNA; see Fig. 4 and Ref. 28). Analogously, although MAP2 protein is typically restricted to dendritic shafts, CaMKIIα and PKMζ proteins are both enriched in synaptodendritic domains, consistent with their proposed functional roles in synaptic signal transduction pathways (47,48).

**Functional Role of PKMζ in the Consolidation of Information Storage**

Activity of PKMζ, an atypical member of the PKC family, is required for the enduring synaptic potentiation that is observed in the maintenance phase of LTP (8). Inhibition of PKMζ (but not of other protein kinases) up to 5 h after tetanization results in the reversal of LTP (8). A functional role as a modulator of synaptic strength is further supported by the recent finding that PKMζ promotes persistence of associative memory in Drosophila (9). These data establish PKMζ as a molecular mediator of long term synaptic plasticity (reviewed in Ref. 10).

How does kinase activity produce changes in synaptic efficacy that are lasting despite the fact that the protein will only have a finite life time at the synapse? New synthesis of kinase protein provides one answer to this question. However, conventional kinases are dependent on activation by second messenger systems. Therefore, increased synthesis following synaptic activation would not directly generate increased kinase activity unless the synaptic stimulation that drives second messenger production was persistent, and second messengers thus produced were continually present. In contrast to conventional kinases, PKMζ is an autonomous and constitutively active kinase that does not require second messenger activation (11,12,49). Therefore, new synthesis of PKMζ during LTP maintenance, i.e. after the period of initial physiological stimulation at the synapse, will result in persistently increased kinase activity; such increased activity would in turn contribute to enduring synaptic enhancement, as has recently been demonstrated (8).

An important consideration in this scenario is input specificity. How can a cell ensure that new PKMζ protein is produced only at synapses that have been activated? We propose that local translational control of PKMζ mRNA at the synapse is critical for the input-specific supply of
PKMζ protein to activated synapses. Our results show that PKMζ mRNA is constitutively transported to, and present in, synaptodendritic domains. In hippocampal neurons, PKMζ mRNA was found colocalized in such domains with dendritic BC1 RNA, a synapse-associated repressor of translation initiation (27). Such colocalization with BC1 RNA is unique to PKMζ mRNA and is not shared with CaMKIIα mRNA. We therefore suggest that in the default state, PKMζ mRNA is present in postsynaptic microdomains but is translationally repressed in a BC1-dependent mechanism. Synaptic stimulation would result in translational derepression and rapid onset of synaptic PKMζ synthesis. Local translational control could also account for the fact that increased PKMζ protein levels are detectable as early as 10 min after tetanic stimulation (13). In summary, therefore, local translational control of PKMζ production is ideally suited to promote enduring consolidation of plastic changes at the synapse. We submit the hypothesis, testable in future work, that although activity of pre-existing PKMζ or other synaptic kinases may suffice to implement short term modifications, on-site production of new PKMζ is required to promote the input-specific and persistent increase in kinase activity that is key to LTP maintenance and other forms of long term memory storage in brain.

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Fig. 1.
Dendritic localization of PKMζ mRNA in hippocampal neurons in primary culture. A–F, substantial labeling signal is observed throughout the dendritic extent. In dark field photomicrographs (A–C), the signal appears as white silver grains over a dark blue background. Corresponding phase contrast photomicrographs are shown in D–F. The signal often appears clustered at branch points and intersections (arrows, B and F). No specific labeling is detectable along axonal shafts (arrowheads in D). G shows a sense strand control. Scale bar, 15 μm (B–D and F) and 40 μm (all others).
Fig. 2. **Dendritic localization of PKMζ mRNA in sympathetic neurons in primary culture.** Labeling signal is evident throughout the dendritic extent. Overall signal levels are about 10 times lower than in hippocampal neurons, partially compensated for by longer exposure times. The signal is robust and clustered in dendrites but is absent from axonal processes. C is a sense strand control. A–C, dark field photomicrographs. D–F, corresponding phase contrast photomicrographs. **Scale bar**, 15 μm (B and E) and 45 μm (all others).
Fig. 3.
Expression of PKMζ mRNA, BC1 RNA, and NSE mRNA in forebrain. PKMζ mRNA (A) and BC1 RNA (B), but not NSE mRNA (C), extend into dendritic layers of CA3-CA1. Note that PKMζ mRNA and BC1 RNA, but not NSE mRNA, are low or absent in dentate gyrus granule cells. Conversely, PKMζ mRNA and BC1 RNA, but not NSE mRNA, are detectable at substantial levels in the hilus. Coexpression of PKMζ mRNA and BC1 RNA was also observed in several other brain areas but not universally in all (a notable exception being the cerebellum). A slight but consistent lateral hemispherical asymmetry is apparent with PKMζ mRNA and BC1 RNA, but to a much lower degree, if at all, with NSE mRNA (for discussion of left-right asymmetry in brain, see Refs. 50 and 51). CA, cornu ammonis; DG, dentate gyrus. Scale bars, 1.2 mm.
Fig. 4. Localization profiles of PKM\textsubscript{ζ} mRNA, BC1 RNA, MAP2 mRNA, and NSE mRNA in the CA3 field of hippocampus. PKM\textsubscript{ζ} mRNA (A), BC1 RNA (B), and MAP2 mRNA (C), but not NSE mRNA (D) are expressed at substantial levels in dendritic layers. PKM\textsubscript{ζ} mRNA and BC1 RNA are expressed at equally high levels in stratum radiatum and in stratum lacunosum moleculare but at lower relative levels in stratum lucidum. In contrast, relative MAP2 mRNA expression levels are highest in stratum radiatum but lower in stratum lacunosum moleculare. The results with all four RNAs were imaged and quantified (E). For each RNA analyzed, relative signal intensities in stratum pyramidale were set at 100%. Or, stratum oriens; Py, stratum pyramidale; Luc, stratum lucidum; Rad, stratum radiatum; LMol, stratum lacunosum moleculare. Scale bar, 250 \(\mu\)m.
Fig. 5.
Design of PKMζ mRNA segments that were used to identify cis-acting DTEs. PKMζ mRNA segments were generated 1) by successive trimming in the 3' to 5' direction (Segments 1–4), 2) by trimming in the 5' to 3' direction (Segments 5 and 6), and 3) by deleting a 42-nt element in the 3'-UTR (Segment Δ).
Fig. 6.  
**Dendritic transport competence of PKMζ mRNA Segment 1.** Segment 1, representing nt 48–1982, is delivered to distal dendritic tips (indicated by *arrows*) in sympathetic neurons in culture. *A–C*, dark field photomicrographs. *D–F*, corresponding phase contrast photomicrographs. *Scale bar*, 50 μm.
Fig. 7.
Dendritic transport competence of PKMζ mRNA Segments 2–4. Segment 2 (nt 48–1898; A and D) and Segment 3 (nt 48–906; B and E) are delivered to proximal but not to distal dendritic domains. Maximal extent of signal in proximal dendrites is indicated by arrows. In contrast, PKMζ mRNA Segment 4 (nt 48–347; C and F) fails to exit the soma to any appreciable extent. A–C, dark field photomicrographs. D–F, Corresponding phase contrast photomicrographs. Scale bar, 50 μm.
Fig. 8.
Dendritic transport competence of PKMζ mRNA Segments 5, 6, and Δ. Segment 5 (1635 nt, lacking 5′ nt 1–347; A and D) is delivered to distal dendrites (arrows) in a manner indistinguishable from Segment 1. In contrast, Segment 6 (1136 nt, lacking 5′ nt 1–846; B and E) fails to enter even proximal dendritic domains. Segment Δ (1893 nt, lacking nt 1905–1946; C and F) is delivered to proximal, but not to distal, dendritic domains (extent of significant labeling indicated by arrows). A–C, dark field photomicrographs. D–F, corresponding phase contrast photomicrographs. Scale bar, 50 μm.
Fig. 9.  
**Dendritic transport competence of PKMζ mRNA: quantitative analysis.** PKMζ mRNA segments used in this analysis are schematically shown in the inset (see also Fig. 5). Relative dendritic signal intensities were established for each PKMζ mRNA segment at various distances along the dendritic extent. In the bar diagram, PKMζ mRNA segments are grouped as follows. Segments 1 and 5 are shown on the left; these segments were transported to the distal-most dendritic domains at substantial levels. Grouped on the right are Segments 2, 3, and Δ; these segments were delivered to dendrites but fail to reach distal domains (>200 μm). Segments 4 and 6 were not exported from the soma (>40 μm) at significant levels and therefore do not appear in the diagram.
Fig. 10.
Secondary structure analysis of putative Mζ DTE2. A 44-nt stable stem-loop structure is predicted for nt 1905–1948 of PKMζ mRNA. Two A-residues (red) are unpaired. The asymmetric A/G bulge (blue ring) and the unpaired A-residues are likely to result in a distinct tertiary structure conformation.