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Polyadenylation-Dependent Translational Control of New Protein Synthesis at Activated Synapse

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Abstract – Synaptic plasticity, which is a long lasting change in synaptic efficacy, underlies many neural processes like learning and memory. It has long been acknowledged that new protein synthesis is essential for both the expression of synaptic plasticity and memory formation and storage. Most of the research interests in this field have focused on the events regulating transcriptional activation of gene expression from the cell body and nucleus. Considering extremely differentiated structural feature of a neuron in CNS, a neuron should meet a formidable task to overcome spatial and temporal restraints to deliver newly synthesized proteins to specific activated synapses among thousands of others, which are sometimes several millimeters away from the cell body. Recent advances in synaptic neurobiology has found that almost all the machinery required for the new protein translation are localized inside or at least in the vicinity of postsynaptic compartments. These findings led to the hypothesis that dormant mRNAs are translationally activated locally at the activated synapse, which may enable rapid and delicate control of new protein synthesis at activated synapses. In this review, we will describe the mechanism of local translational control at activated synapses focusing on the role of cytoplasmic polyadenylation of dormant mRNAs.

Key words ☐ Synaptic plasticity, polyadenylation, cytoplasmic polyadenylation element binding protein (CPEB), translation, RNA binding protein

Synaptic Plasticity

Cognitive deficit and learning impairment is one of the hallmark features of several forms of neurological diseases such as Alzheimer's disease (AD) and Fragile X mental retardation syndrome (FXS). To gain a better control of the mental retardation and age-related cognitive deficit, it is crucial to understand the mechanisms underlying learning and memory. It is more than obvious that synapses are basic units of neural information processing. Synapses utilize various structural and biochemical strategies to efficiently transmit a signal from an input to a target. Synapses are also equipped with biochemical and morphological methods to adjust their signaling properties according to the previous history of neural stimulation. This use-dependent changes in synaptic efficacy; also called synaptic plasticity is a basis of information processing and storage in the brain. The synaptic plasticity has two different temporal components. One is short-term synaptic plasticity, which occurs within milliseconds to minutes after appropriate synaptic stimulation and lasts only transiently at best up to 1-2 hours. While this type of synaptic plasticity does not require new protein synthesis at the activated synapse, which means the modification of existing protein is sufficient to mediate the required synaptic changes, the other type of synaptic plasticity called long-term synaptic plasticity is essentially dependent on the new protein synthesis. Long-term synaptic plasticity lasts days to years and maybe during the last of the life of an individual (Lisman and Fallon, 1999) and is generally accepted as a mechanistic basis for the memory storage in the brain.

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New protein synthesis at activated synapses

Regarding the protein synthesis dependency of long-term synaptic plasticity, several immediate questions are arising. For example, what are the proteins newly synthesized after synaptic

stimulation and what is the role of those proteins in establishing and maintaining synaptic plasticity? Another obvious question is the mechanism of the regulation of new protein synthesis at the activated synapse. This is very important considering the highly polarized structure of mammalian neurons. There exist approximately ten thousands synapses per average excitatory neurons and in extreme cases one hundred thousands synapses in neurons like cerebellar Purkinje cells. How a neuron can achieve specificity of new protein synthesis only at the activated synapses among tens of thousands of other inactive synapses? Neurons utilize at least three strategies for the activity dependent regulation of protein synthesis and targeting. First, some proteins are translated in the soma from newly transcribed mRNAs. For example, transcription factors such as CREB (cAMP-response-element-binding protein) and C/EBP (CCAAT enhancer binding protein) are activated in response to particular forms of synaptic stimulation (Alberini *et al.*, 1994). The mechanism to regulate the transport of newly synthesized proteins to a remote synaptic site is not fully understood yet but several researches suggest the involvement of the formation of a kind of "tag" at the activated synapse to specifically recruit the newly synthesized proteins (Frey and Morris, 1997). Second, newly transcribed mRNAs may be transported to activated synapses, where they are translated. This mechanism has been recently described for *Arc*, an immediate-early gene whose transcription is tightly regulated by synaptic activity (Steward *et al.*, 1998). It has been also reported that the transport of a-CAMKII mRNA to activated synapses increased after neural stimulation both in mammals and Drosophila (Rook *et al.*, 2000; Ashraf *et al.*, 2006), which is regulated by specific cis-element(s) in its 3'-UTR. It is also acknowledged that the transport of mRNA containing granules to dendritic spine is facilitated by neural activity (Krichevski and Kosik, 2001). The supply of mRNAs into the dendritic region is presumably accompanied by concomitant protein synthesis upon their arrival at the appropriate target synaptic region. The third mechanism regulating the synaptic protein synthesis is the regulation of protein translation using mRNAs existing at the synapse before the arrival of synaptic activation. This hypothesis is originated from the fact that almost all the constituents required for the protein translation such as mRNA, tRNA, ribosome, initiation and elongation factors and even satellite protein secretory pathways are localized in and around the dendritic spine (Steward and Levy, 1982; Tiedge and Brosius, 1996; Kleiman *et al.*, 1990; Pierce *et al.*, 2001). Several different family members of RNA binding proteins have been also reported to exist at synapses including fragile mental retardation protein (FMRP), staufen and cytoplasmic polyadenylation binding proteins (CPEB), which might be important in the regulation of mRNA transport, stability and translational efficiency. The local regulation of new protein synthesis from existing mRNAs has temporal and spatial advantages compared with global control of new protein synthesis combined with specific targeting. Actually, it has been suggested to be critical in protein synthesis dependent phase of long-term synaptic plasticity (Kelleher *et al.*, 2004; Klann *et al.*, 2004).

Mechanism of translational control of new protein synthesis in neuron

Even though there are considerable recent advances in this field, the mechanisms mediating local translational control of dormant mRNAs at activated synapses are not clear yet. In general, regulation of new protein translation is achieved at the level of translational initiation. The initiation of eukaryotic translation of mRNA is subdivided into three steps. First, initiator $tRNA^{Met}$, eukaryotic initiation factor eIF2a and GTP bind to form ternary complex. The ternary complex associates with eIF3-40S ribosomal subunit, which completes the formation of 43S pre-initiation complex. Second, the eIF4F groups of factors bind to the m^7G-cap of a candidate mRNA and in conjunction with eIF4B, join the 43S complex to form the 48S pre-initiation complex. The 48S initiation complex scan target mRNA and locate initiation codon AUG. Third, eIF5 hydrolyses the ternary complex GTP, releasing other initiation factors and allowing binding of the 60S large ribosomal subunit to form the functional translation complex, which is ready for elongation of polypeptide chain (Fig. 1).

eIF2α, which is involved in ternary complex plays important

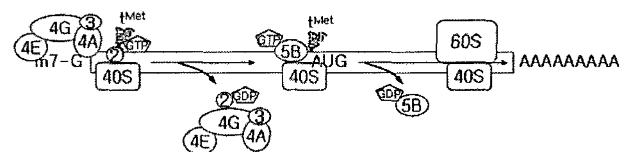

Fig. 1. The ternary complex, eIF3 and cap binding complexes (eIF4E, 4G and 4A) associates together with 40S ribosomal complex to form the 43S initiation complex. The 43S initiation complex scans along the mRNA until it reaches to initiator AUG codon. Initiation factors are released from the complex and 60S ribosomal complex is recruited to form elongation-competent 80S complex. This is simplified and schematic representation of translational initiation process (adapted from Mendez and Richter, 2001).

role in the regulation of translation. Several kinases including the heme-regulated eIF2α kinase (HRI), the PKR-like endoplasmic reticulum eIF2α kinase (PERK), the amino acid-regulated eIF2α kinase (GCN2) and the RNA-dependent eIF2α kinase (PKR) can phosphorylate eIF2α, which leads to the inhibition of GDP-GTP exchange reaction mediated by eIF2B. Resulting decrease in the level of GTP bound form of eIF2 restricts the association of ternary complex with other initiation factors. In cultured neuron, application of BDNF reduced the phosphorylation of eIF2α, which might be related to the increased new protein synthesis and BDNF-induced synaptic plasticity (Takei *et al.*, 2001). Another point of regulation is cap binding complex, especially, eIF4E. Huber et al. reported that mGluR dependent LTD is blocked by the addition of a 5'-cap analog m7GpppG, which dissociates eIF4E binding to 5'-cap of target mRNAs (Huber *et al.*, 2001). Several signaling pathways mediate phosphorylation of eIF4E, which generally increase translational efficiency. Neural stimulation by L-LTP inducing stimuli, NMDA receptor activation and BDNF treatment induces Mnk1 kinase activation via Erk activation, which phosphorylates eIF4E. It has been also suggested that activation of p38 kinase in Aplysia by serotonin increases eIF4E phosphorylation. The binding of eIF4E to 5'-cap of mRNAs is regulated by a family of proteins called 4E binding proteins (4E-BPs). 4E-BPs bind and sequester eIF4E from 5'-cap binding, thereby reduce translational efficiency. The phosphorylation of 4E-BPs, mediated by mammalian target of rapamycin (mTOR) pathway, decreases the binding affinity of 4E-BPs toward eIF4E resulting in the translational activation. Treatment of rapamycin, which blocks the activation of mTOR pathway, prevents L-LTP, BDNF-induced potentiation and mGluR-mediated depotentiation of LTP in hippocampus (Tang *et al.*, 2002; Zho *et al.*, 2002; Cammalleri *et al.*, 2003). mTOR and Erk pathway also mediate the phosphorylation of S6 kinase, which mediates phosphorylation and activation of ribosomal S6 protein. Activation of ribosomal S6 protein increases translation of a subset of mRNAs containing 5'-terminal oligopyrimidine tract (5'-TOP). L-LTP increases both S 6 kinase activation and S6 protein phosphorylation, which might be related to the increased synthesis of ribosomal components having 5'-TOP sequences in their mRNAs.

The above mentioned regulatory mechanisms apply to the global control of translational regulation, which regulates translation of almost all or at least large subsets of mRNAs. Relatively little is known about the regulatory mechanism for the translational control of specific small subsets of mRNAs. During germ line and larval development, translation of cell cycle regulators and certain transcriptional factors is regulated by the binding of RNA binding proteins to 3' or to a lesser extent to 5'-UTR sequences of mRNAs. In neurons, several RNA binding proteins has been shown to bind 3' or 5'-UTR sequences of specific subsets of mRNAs and regulate mRNA transport, stability and translation. The importance of the translational control by these mRNA binding proteins in the regulation of neural development and synaptic function is most well exemplified in fragile X mental retardation syndrome (FXS). FXS is a neurodevelopmental disorder with mental retardation. FXS is caused by the trinucleotide repeat expansion mutation in FMR1 gene, which leads to the termination of expression of FMR1 gene product FMRP (Antar and Bassell, 2003). Interestingly, FMRP is a RNA binding protein and acts as a translational repressor. The absence of FMRP may lead to the abnormal translation of target mRNAs, which might be important in the regulation of mGluR-mediated LTD as well as the formation and maintenance of synaptic contacts. However, the molecular details of the mechanism that FMRP regulates the translation of its target mRNAs are not clear yet. The most well known molecular mechanism of translational control by a RNA binding protein is the regulation of the length of poly A tail of mRNAs by CPEB, which is important in the regulation of synaptic plasticity as well as memory process (Berger-Sweeney *et al.*, 2006; Alarcon *et al.*, 2004).

CPEB dependent polyadenylation of mRNAs and new protein synthesis at synapses

The most well known molecular mechanism regulating local translation of new protein synthesis at activated synapses is CPEB dependent, polyadenylation-induced translation of target mRNAs. The regulation of mRNA translation by CPEB is first known in germ cells (Richter, 2001; Wells *et al.*, 2000). In the *Xenopus* oocyte, progesterone stimulation leads to phosphorylation of xCPEB by Aurora-A kinase and translation of CPE containing mRNAs such as cmos, cyclin B1 and cdk2, which lead to the proper cell cycle progression (Stebbins-Boaz et al., 1999). In the Xenopus oocyte, the length of poly A tail for these mRNAs is kept relatively short (10-40 nucleotides long) and these mRNAs are translationally dormant. It is only after the elongation of poly A tail to more than 150 nucleotides long when translation of these mRNAs begins followed by oocyte maturation. Polyadenylation requires two elements in the 3 UTR. The first is hexanucleotide sequence AAUAAA (poly-

adenylation signal), which is also necessary for nuclear pre-mRNA cleavage and polyadenylation. The second element is cytoplasmic polyadenylation element (CPE), which should be localized in close proximity to hexanucleotide sequence (usually within 20-30 nucleotides and at most 100 nucleotides, for a review see Mendez and Richter, 2002). The general consensus sequence of CPE seems to be UUUUUAU but CPE is not very stringent and may include sequences as diverse as UUUUAU to UUUUAACA. Some mRNAs (for example, cyclin B1) contain many CPEs, which might be related to the ability and kinetics of polyadenylation after stimulation. Overall, the precise sequence of the CPE, the number of copies of the CPE, the distance between the CPE and the hexanucleotide, or sequences adjacent to the CPE might regulate the time at which polyadenylation takes place. After progesterone stimulation, CPEB is phosphorylated by a protein kinase called Aurora (Mendez et al., 2000a), which induces the interaction of cleavage and polyadenylation specificity factor (CPSF) to hexanucleotide sequence followed by the binding of cytoplasmic poly A polymerase to lengthen poly A tail and increase the recruitment of ribosome to the mRNA resulting in the increased translation (Mendez *et al.*, 2000b). In the resting status, CPEB is bound to maskin, which is similar to 4E-BP in that it interacts with eIF4E and inhibits the interaction of eIF4E with eIF4G. After phosphorylation on CPEB, maskin dissociates from eIF4E allowing the interaction of eIF4E with eIF4G. It is believed that the interaction between 5'-cap and poly A tail increases translational efficiency perhaps via the formation of closed loop between 5' and 3'-UTR. Circular mRNA may have advantage to recycle ribosome from 3'-UTR side to 5'-UTR (i.e. initiation region) side of the mRNA after finishing one round of translation. Cytoplasmic poly A tail binding protein (PABP) can bind to poly A tail as well as eIF4G. Maskin dissociation and polyadenylation allow efficient eIF4G and PABP binding to 5' and 3'-UTR side each resulting in the formation of closed circular structure via eIF4G and PABP interaction, which explains increased translational efficiency (Fig. 2).

Even though initial studies did not reveal significant expression of CPEB outside ovary and testis, it is clear now that CPEB is expressed in moderate level in brain tissue. CPEB is present in hippocampus as well as cerebellum and cortex and localized at synapse, especially in dendritic spine. CPEB is also co-fractionated with postsynaptic density. It raised interesting possibility that CPEB regulates translation of synaptic proteins in postsynaptic compartment affecting synaptic plasticity. In addition to CPEB, maskin, PABP, cytoplasmic PAP and Aurora

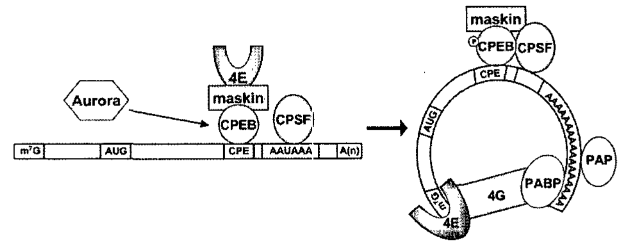

Fig. 2. Schematic representation of the mechanism of CPEB-dependent polyadenylation. CPEB binds to maskin and CPE sequence of 3'-UTR of target mRNA. Binding of maskin to eIF4E prevents eIF4G binding to eIF4E. After appropriate signal, Aurora kinase phosphorylates CPEB, which leads to the binding of CPSF and PAP resulting in the increased polyadenylation. Dissociation of maskin allows the binding of eIF4G to eIF4E at 5'-UTR and increased binding of PABP to long poly A tail results in circular configuration of mRNA by eIF4E-eIF4G-PABP interaction, which has increased translational activity (adapted from Klann *et al.*, 2004).

kinase is expressed in hippocampal neuron and localized at synapses. In hippocampal neurons, CPEB1 regulates the synthesis of the a-subunit of calcium/calmodulin dependent kinase II (α-CaMKII) following glutamate receptor activation (Huang *et al.*, 2002; Wells *et al.*, 2001; Wu *et al.*, 1998). The a-CaMKII mRNA contains two conserved CPE element in its 3'-UTR and the translation was induced following the activation of the N-methyl-D-aspartate (NMDA) type glutamate receptor (Wells *et al.*, 2001). The increase in translational efficiency is mediated by polyadenylation i.e. treatment of the polyadenylation inhibitor cordycepine completely abolished glutamate-induced polyadenylation and increased translation. In addition to the increased polyadenylation and translation, the binding of CPEB to CPE sequences of 3'-UTR of a-CaMKII mRNA is essential for the localization of the mRNA at dendritic compartment (Huang *et al.*, 2003). These results suggest that both mRNA transport and translational activation is mediated by CPEB in hippocampal dendrites. Similar to the situation in oocyte, it has also been shown that NMDA stimulation activates Aurora kinase in the hippocampal neuron. In addition to the Aurora-A kinase, Soderling group have recently shown that α-CaMKII is capable of phosphorylating CPEB on a site critical to its activation (T^{171}, S^{174}), which leads to CPEB-dependent protein-synthesis (Atkins et al., 2004). The same group of authors also suggested that the balance between α-CaMKII and protein phosphatase 1 (PP1) activity is important during hippocampal LTP induction (Atkins *et al.*, 2005). This suggests that independent mechanisms can regulate CPEB-mediated

protein synthesis in the same cell. The signaling pathway leading to the Aurora kinase activation is not evident yet. Recent studies suggested that GSK3b pathway is involved in the activation of Aurora kinase at least in oocyte. GSK-3β phosphorylates Aurora A on S290/291, the result of which is an autophosphorylation of serine 349. GSK-3β phosphorylated Aurora A have reduced or no capacity to phosphorylate CPEB (Sarkissian *et al.*, 2004). Progesterone stimulation in oocytes inhibits GSK-3β activity, resulting in the increased Aurora A activity. GSK3β pathway is regulated by PI3 kinase pathway and this raises interesting possibility that mTOR or mGluR activation cross-talks to NMDA receptor stimulation at the level of GSK3b and Aurora kinase activation.

Physiological significance of polyadenylation-dependent translational control

One of the most important issues in the polyadenylation dependent regulation of synaptic translation is the physiological significance of such regulatory mechanism. Several recent studies have shown the importance of CPEB for synaptic plasticity. In Aplysia model of synaptic plasticity, serotonin stimulation induces an increase in CPEB levels, which is both mTOR and PI3K dependent (Si *et al.* 2003). In addition, CPEB antisense nucleotides blocked LTF, indicating that CPEB is required for the stabilization of LTF (Si *et al.* 2003). CPEB also appears to be required for normal mammalian synaptic plasticity. CPEB-1 gene knockout mice have impaired LTP and LTD (Alarcon *et al.* 2004). These findings suggest that synaptically localized mRNA containing CPE sequences may be likely target of translational regulation during the induction of synaptic plasticity. More recently, Richter group have shown that CPEB1 KO mice have reduced extinction of hippocampus dependent memory along with the changes in hippocampal gene expression (Berger-Sweeney *et al.*, 2006).

During development, light exposure induces massive synaptic activation in the visual cortex that leads to functional and structural synaptic plasticity. In such paradigms, light stimulation induced rapid, synaptic polyadenylation of mRNA encoding α-CaMKII followed by new protein synthesis of α-CaMKII by a translational mechanism (Wu *et al.*, 1998). This suggested a role for CPEB in synaptic plasticity in vivo. More recently, we observed that the translation of tissue plasminogen activator (tPA), which is very important in the manifestation of synaptic plasticity such as LTP and LTD as well as acquisition and maintenance of memory, is increased by polyadenylation-dependent manner (our unpublished results). Altogether, these results suggest that CPEB dependent polyadenylation of dormant mRNAs at activated synapses plays important role in the regulation of synaptic plasticity both in vitro and in vivo.

Target mRNAs of CPEB dependent translational control.

One of the most important questions regarding CPEB dependent local translational control in neuron is the identification of the complement of the mRNAs regulated by CPEB and delineation of its function at activated synapses. The cis-elements where CPEB can bind are composed of CPE (UUUUUAU) and polyadenylation signal (AAUAAA). The distances between CPE and hexanucleotide sequences confer additional constraint for the CPEB binding. Those two elements should be within 100 nt from each other (Mendez *et al.*, 2002), even though it is not clear whether physical or spatial proximity is more important. Bioinformatic searches based on these criteria revealed hundreds of candidate target mRNAs, which are important in synaptic transmission, structural organization, cell to cell interaction, migration, apoptosis and cell cycle regulation (Table I). The *in silico* search of the target mRNAs should be verified by experimental demonstration of direct binding and regulation by CPEB. In addition, the target mRNAs should be localized at dendritic compartment so that it can play regulatory role during synaptic activation. Several researchers reported at least 400 different mRNAs are localized at synaptic compartment. For example, we isolated RNAs from crude homogenate and biochemical preparation of synapses called 'synaptoneurosome'

Table I. Candidate CPE-containing mRNAs (CPE-like sequence; HEX wequence)

	Sp.	Accession	3'-UTR sequence	Function
Abi1*	Mus	BC004657	UUUUUAUU-59nt-UUUUAU-6nt-UUUUAUAAUAAA	actin poly.
IRSp53*	Mus	AB105196	UUUUUUAU-17nt-AAUAAA	actin poly.
β-catenin*	Mus	NM_007614	UUUUAAU-67nt-AUUAAA-1nt-UUUUAAUU	signaling
pleiotrophin	*Rat*	*NM_017066*	*UUUUUAUU-72nt-UUUUUAAU-28nt-AAUAAA*	*cell adhesion*
RPTP β/ζ	Rat	U09357	UUUUUAU-34nt-UUUUUAU-15nt-AAUAAA	cell adhesion
p120 catenin	Mus	BC054544	UUUUUAU-3nt-AAUAAA	cell adhesion
EB1*	Mus	NM_007896	UUUUAUU-56nt-UUUUUAAAUAAA	MT binding
Tiam 1	Mus	U05245	UUUUAU-18nt-UUUUUAU-43nt-AAUAAA	Rac-GEF
Cyclin B1*	Mus	NM_172301	UUUUAAU-22nt-AAUAAA	Cell cycle
Cyclin B2	Mus	BC008247	UAUUUUAU-58nt-AAUAAA	Cell cycle
Cyclin L1	Mus	NM_019937	UUUUUAU-108nt-AAUAAA-12nt-UUUUUAU	Cell cycle
Cyclin G2	Mus	AF005885	UUUUUAUU-72nt-AAUAAA	Cell cycle
SKP2*	Mus	BC092236	UUUUAU-13nt-AAUAAA-4nt-UUUUUAU	Cell cycle
cdc20*	Mus	NM_023223	UUUUUAUUUUAU-7nt-AAUAAA	Cell cycle
Cdk2*	Rat	BC061832	UUUUUAU-19nt-UUUUAU-56nt-AUUAAA	Cell cycle
Thrombo-spondin*	Mus	BC050917	UUUUUAU-10nt-UUUUUAU-62nt-AAUAAA	ECM/synapse formation

*Similar sequence detected in homologous protein in at least one other species including human.

Partial list of candidate mRNAs for CPEB dependent translational regulation. Many mRNAs are involved in cell to cell interaction, cell cycle regulation and synapse formation. Many other candidates not shown here are mRNAs whose translational products are important in synaptic signal transduction and regulation of cell survival and death (Data is from Dr. David Wells, personal communication).

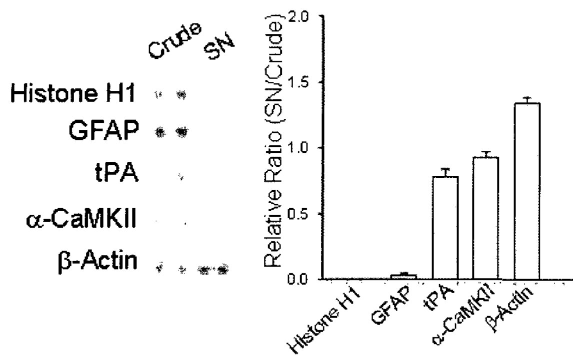

Fig. 3. Presence of tPA mRNA in the synaptic fraction. mRNA level was determined by RT-PCR from crude homogenate and synaptoneurosome (SN) fraction from rat hippocampus. The mRNAs for tPA, α-CaMKII and β-actin are relatively enriched in the SN fraction compared to non-dendritically localized mRNAs for Histone H1 and GFAP. A ratio of the SN band intensity divided by the level in the crude homogenate is plotted on the right (adapted from Shin *et al.*, 2004).

and analyzed by RT-PCR for the existence of several mRNA species. Interestingly, mRNAs encoding α-CAMKII and tPA are relatively enriched at synapses, which contain CPE sequences as well. However, those mRNAs encoding nuclear protein histone or glial cell marker protein GFAP are not enriched at synapses (Fig. 3).

The most widely studied candidate target mRNA for CPEB dependent translational regulation is α-CAMKII mRNA. In addition, an Aplysia mRNA encoding N-actin appears to be regulated by polyadenylation during synaptic plasticity. N-actin mRNA contains a CPE in its 3'-UTR. The poly A tail of N-actin RNA has been shown to be elongated in response to stimulation with serotonin (Si *et al.*, 2003). Recently, we have shown that another mammalian mRNA encoding tPA is regulated by CPEB- and polyadenylation-dependent translational control (Shin *et al.*, 2004). tPA mRNA contains two conserved CPE sequences and a hexanucleotide sequence in its 3'-UTR. tPA knock out mice or the treatment of tPA inhibitor prevented late phase long-term potentiation (LTP), which is a widely accepted model of long term synaptic plasticity. Likewise, the over-expression of tPA increased L-LTP. tPA is also involved in long term depression (LTD) induction, which is another well-known model of long-term synaptic plasticity. A high concentration of tPA is toxic to neuron and it is rapidly released from neuron after stimulation, which suggests the local concentration of tPA at activated synapse should be rigorously regulated. In addition, an unidentified protein regulates polyadenylation and translation of tPA mRNA in mouse oocytes. These results imply that tPA is an ideal target for the CPEB dependent translational control at activated synapses. We found that Immunoreactivity of tPA is localized in dendrite. Some of the immunoreactive puncta co-localized with synaptic marker protein synaptophysin, which suggest that tPA is localized in dendrite as well as in axon and some of the dendritic tPA is localized at the synapse presumably postsynaptically. In addition to the protein localization, indirect fluorescent in situ hybridization (FISH) using probes specific for tPA mRNA has shown specific tPA mRNA signals in dendrite. The signal density was usually high in dendritic branch point, which might suggest that the transport of tPA mRNA along the dendrite is a regulated process. When cultured primary rat hippocampal neurons were transfected with green fluorescent protein labeled tPA (GFP-tPA), transfected tPA was localized in dendrite as a punctate pattern consistent with the immunocytochemistry results, which might suggest that tPA is localized within transport and/or secretory granules. Stimulation of the cultured neuron and isolated synaptoneurosome fraction rapidly increased tPA level, which is dependent on translation but not transcription. Interestingly, polyadenylation inhibitor cordycepin inhibited the increase of tPA level. Unlike to α-CAMKII, the polyadenylation and increased tPA translation is dependent on mGluR stimulation, which suggests both NMDA and mGluR pathway can regulate CPEB dependent translation depending on the context of stimulation and species of target mRNAs. We further examined the association of tPA mRNA with CPEB. Recombinant glutathione S-transferase (GST) fused CPEB was mixed with brain lysate and full down with glutathione-agarose. tPA mRNA but not negative control mRNA encoding glial fibrillary acidic protein was recovered from precipitate. Immunoprecipitation using an antibody specific against CPEB also gave similar recovery of tPA mRNA but not GFAP mRNA. With the combination of more powerful and systemic approach like microarray, these methods would be useful to determine the full constituents of CPEB target mRNAs from brain.

Concluding remarks

Identifying mRNA targets for local synaptic translational control and understanding the mechanism of their translational regulation will contribute not only to the acquisition of better information for the mechanisms regulating synaptic plasticity but also to the development of methods to modulate the learning and memory impairments both in pathological and normal aging situations. In addition to Aurora kinase and α-CAMKII,

the identification of the involvement of other kinases and phosphatases in the regulation of CPEB dependent polyadenylation as well as the understanding of the signal transduction pathway may provide pharmacological tools designed to regulate synaptic functions. The involvement of CPEB in oocyte development and differentiation as well as in the regulation of embryonic cell cycle (Groisman *et al.*, 2002) suggests possible role of polyadenylation dependent translation control in glial cell proliferation (and maybe activation process as well) in pathological conditions and neuronal and glial differentiation from neural stem cells. Over-expression of some RNA binding proteins leads to the apoptosis presumably by the perturbation of the regulated expression of apoptotic and anti-apoptotic proteins. Therefore, the involvement of polyadenylation-dependent translational control in neurodegenerative condition will be yet another interesting field to be examined in the future.

Translational control of protein expression in the brain has long been ignored relative to the transcriptional control mechanism. With the benefit of the possibility of rapid and local control of the regulation of gene expression, understanding polyadenylation-dependent translational control mechanism in the brain may provide myriads of new possibility for the fine-tuning of neural functions.

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