Journal Club

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A TOP at the Synapse

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Review of Huang et al. (<http://www.jneurosci.org/cgi/content/full/25/31/7199>)

5'TOP mRNA species contain a short 5' Tract rich in OligoPyrimidines that encode for translation factors and vertebrate ribosomal protein subunits. Because 5'TOP mRNAs are rapidly translated, they are attractive candidates for dendritically localized molecules that could enhance protein synthesis in activated synapses, leading to the expression of long-term plasticity. The 5'TOP-encoded protein eukaryotic elongation factor 1A (eEF1A, also known as EF1 α) is an essential factor that joins aminoacyl-tRNA to the ribosome during translation. Interestingly, eEF1A performs many extratranslational functions including the binding of mRNA transcripts and bundling of filamentous actin (F-actin) (Liu et al., 2002). Notably, eEF1A mRNA is transported and docked to activated synapses during long-term facilitation in *Aplysia*, and the synthesis of eEF1A coincides with the maintenance phase of plasticity (Giustetto et al., 2003). Undoubtedly, this inspired the authors, in their *Journal of Neuroscience* article (<http://www.jneurosci.org/cgi/content/full/25/31/7199>), to characterize the localization of eEF1A transcript and protein in models of mammalian synaptic plasticity.

The authors began their studies by examining the basal expression of eEF1A mRNA in the brain. eEF1A transcript was observed to be ubiquitously expressed. Al-

though neuronal somata were highly enriched, only specific dendritic regions expressed moderate levels of the transcript [Huang et al. (2005), their Fig. 3 (<http://www.jneurosci.org/cgi/content/full/25/31/7199/FIG3>)]. In contrast to other known dendritic mRNAs, a developmental decline of eEF1A transcript levels in synaptic terminals was observed. This suggests that eEF1A mRNA is restricted to subserve specific types of synaptic activation in the developing and adult brain.

To evaluate eEF1A transcript distribution during synaptic activation, a long-term potentiation (LTP)-inducing protocol known to cause induction and subsequent transport of Arc mRNA, a dendritically translated transcript, to the middle molecular layers of the dentate gyrus (DG) was used. Although changes were described for Arc, no alterations in eEF1A transcripts could be detected in the DG [Huang et al. (2005), their Fig. 4 (<http://www.jneurosci.org/cgi/content/full/25/31/7199/FIG4>)]. Similarly, treatment of dendritic laminae with the group I metabotropic glutamate receptor (mGluR) agonist (*R,S*)-3,5-dihydroxyphenylglycine (DHPG), which induces long-term depression (LTD), did not alter eEF1A transcript distribution. It is important to consider that the *in situ* hybridization techniques used in these experiments are appropriate for the detection of robust signals, and that, as the authors acknowledge, microcellular changes in eEF1A transcript targeting below the threshold of detection may have occurred. Nevertheless, these findings demonstrate that prominent transcription and/or migra-

tion of eEF1A transcripts does not occur in these models of synaptic activation.

The authors next determined whether eEF1A protein levels were affected. DHPG applied to the dendritic laminae increased local immunostaining for eEF1A that could be blocked by previous injection of anisomycin, an inhibitor of protein synthesis. These results were recapitulated *in vitro* with DHPG-treated hippocampal slices followed by Western blotting for eEF1A. However, the entire hippocampus, including somata highly enriched in eEF1A mRNA, was assayed *in vitro*. Perhaps isolation of synaptoneurosomes from DHPG-treated slices would more accurately quantify dendritic eEF1A protein elevation.

In contrast to the protein synthesis-dependent enhancement of eEF1A associated with DHPG-LTD, existent eEF1A protein appeared to redistribute to activated synapses. During LTP, the immunoreactivity of a well defined band of eEF1A in the middle molecular layers of the DG could be observed in conjunction with a noticeable depletion of eEF1A immunoreactivity in the cell body layer [Huang et al. (2005), their Fig. 7 (<http://www.jneurosci.org/cgi/content/full/25/31/7199/FIG7>)]. Surprisingly, discrete application of either anisomycin or rapamycin (which blocks 5'TOP synthesis) in the DG did not affect the appearance of this eEF1A band, whereas infusion of latrunculin, an inhibitor of F-actin formation, blocked the expression of eEF1A specifically where F-actin was depleted [Huang et al. (2005), their Fig. 9 (<http://www.jneurosci.org/cgi/content/full/25/31/7199>)].

Received Aug. 25, 2005; revised Sept. 17, 2005; accepted Sept. 17, 2005.

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DOI:10.1523/JNEUROSCI.3612-05.2005

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FIG9)]. These results are in contrast to those of Tsokas et al. (2005), who demonstrated that LTP in area CA1 promotes eEF1A protein synthesis in isolated dendrites that is blocked with either anisomycin or rapamycin. Importantly, the *in vivo* LTP protocol used by the authors and the *in vitro* method of Tsokas et al. (2005) were performed in different regions of the hippocampus and monitored over different temporal windows, potentially explaining the different observations.

There are important questions raised by these studies. First, does the newly synthesized eEF1A observed in DHPG-treated dendrites facilitate local increases in translational capacity? The authors attempted to answer this question by measuring [^3H]leucine incorporation during dendritic DHPG application and found no appreciable enhancement [Huang et al. (2005), their Fig. 6 (<http://www.jneurosci.org/cgi/content/full/25/31/7199/FIG6>)]. An observation of large dendritic changes in ^3H incorporation would have provided important evidence consistent with enhanced translational capacity. However, because polyribosomes and translation machinery are sparsely distributed in dendrites, it is unclear whether elevations of dendritic translational capacity are detectable by this autoradiographic approach. A more sensitive method to measure the basal and maximal protein synthetic capacity of dendrites is required to evaluate the impact of newly formed eEF1A in these subcellular compartments. One approach would be to measure the incorporation of [^{35}S]methionine in DHPG-treated synaptic preparations, a method which has been reported by Raymond et al. (2000) in whole hippocampal slices to enhance *de novo* synthesis by ~17%.

Second, does the eEF1A observed in F-actin-rich synapses facilitate translation of specific mRNAs? Although the authors have shown that it is unlikely that eEF1A transcript is itself translated during LTP, the prominent redistribution of eEF1A to areas enriched by F-actin suggests that eEF1A is poised to promote the local synthesis of mRNAs docked in close apposition to the cytoskeleton and ribosomes.

Figure 1. Models of synaptically induced regulation of eEF1A. DHPG-LTD: (1) mGluR activation triggers the rapid dendritic translation of eEF1A. (2) Newly available eEF1A may allow translation of more transcripts and influence protein synthetic capacity. LTP: (3) eEF1A binds to and bundles F-actin that accumulates to stimulated synapses. (4) eEF1A also binds to transcripts and may influence their translation near the actin cytoskeleton.

To address this issue, it would be interesting to see whether β -actin mRNA, a binding partner for eEF1A, is dendritically translated during LTP.

Huang et al. (2005) are the first to demonstrate that a TOP-encoded protein is differentially regulated in synaptic plasticity. In the case of DHPG-LTD, eEF1A mRNA is locally translated, whereas in the case of LTP, existent eEF1A protein is relocalized to F-actin-rich synapses (Fig. 1). Intriguingly, these two distinct functions of eEF1A may parallel the finding that LTP- and LTD-inducing stimuli have opposing effects on the dynamic regulation and stabilization of the actin cytoskeleton in synapses (Okamoto et al., 2004). This suggests that diverse mechanisms involving eEF1A promote appropriate changes in synaptic strength to ongoing stimuli.

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