Journal Club

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Visualizing Local Protein Synthesis and Its Modulation by FMRP and Visual Experience

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Review of Liu and Cline

Protein synthesis is required to support long-term experience-dependent changes in behavior (Flexner et al., 1963). Synaptic activity recruits transcriptional and translational machinery that alters gene expression, supporting long-term synaptic plasticity and structural remodeling necessary for behavioral changes (Mayford et al., 2012). However, to specifically adjust the information flow in a neural network, newly synthesized proteins need to be present at precise subcellular locations of the neuronal architecture (Martin and Zukin, 2006). One of the proposed mechanisms by which proteins are localized to specific sites is local translation (Sutton and Schuman, 2006). Studies *in vitro* have provided evidence for local translation in dendrites (Kang and Schuman, 1996; Huber et al., 2000); however, results from *in vivo* preparations remain scarce.

Regulation of local protein synthesis can be achieved by RNA-binding proteins that control the location and timing of

mRNA translation (Holt and Schuman, 2013; Klein et al., 2016). Fragile X mental retardation protein (FMRP) is one such RNA-binding protein that controls translation of a specific subset of mRNAs (Darnell and Klann, 2013). A CGG expansion of the *FMR1* gene leads to Fragile X syndrome (FXS), the most common inheritable intellectual disability (Bagni and Greenough, 2005). Rodent models of FXS exhibit abnormal synaptic plasticity and long-term memory impairments (Huber et al., 2002; Zhao et al., 2005). Yet the mechanism of FMRP action *in vivo* remains unclear. In a recent article in *The Journal of Neuroscience*, Liu and Cline (2016) address this question by investigating the function of FMRP in local protein synthesis and behavioral plasticity in *Xenopus laevis*.

Liu and Cline (2016) developed a protocol using Fluorescent Noncanonical Amino Acid Tagging (FUNCAT) (tom Dieck et al., 2015) to visualize newly synthesized proteins in *Xenopus* optic tectum. The transparency of *Xenopus laevis* and spatial and temporal optimization of FUNCAT allowed the authors to assess translation across different layers of the intact optic tectum, including the neuropil, a region exclusively populated by axons and dendrites. To validate the sensitivity of their approach, the authors demonstrated that blocking new protein synthesis with translation blockers decreased FUNCAT signal in the tectum,

whereas increasing neural activity by blocking GABAergic transmission enhanced FUNCAT signal. These results suggest that FUNCAT is a sensitive tool for *in vivo* measurements of newly synthesized protein.

To selectively examine local translation, colchicine was used to block microtubule polymerization to prevent protein trafficking from the soma. Although colchicine did not disrupt translation, it interfered with mitochondrial trafficking. Using this strategy, the authors next asked whether the FUNCAT protocol could be used to detect changes in local translation as a result of behavior. They used a visual conditioning protocol in which tadpoles are exposed to 1-cm-wide white bars on a dark background moving in each of the four cardinal directions in random order. This training method induces plasticity of the avoidance behavior, defined as a change in swim direction when an object approaches the tadpole eye at an approximate right angle. This plasticity is protein synthesis-dependent and requires cytoplasmic polyadenylation element binding protein (CPEB) (Shen et al., 2014). In *Aplysia*, CPEB has been reported to regulate local translation in the context of synaptic plasticity (Si et al., 2003). However, whether local translation is part of the mechanism underlying behavioral plasticity in *Xenopus* is unknown. Liu and Cline (2016) detected an increase in local translation within the tectal neuropil fol-

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lowing the visual conditioning protocol, suggesting that visual experience can regulate local translation in an intact brain area (Liu and Cline, 2016).

The authors next tested whether FMRP plays a role in local protein translation and behavioral plasticity, the ability to adjust behavior in response to the changing environment. Using morpholinos to knock down FMRP, the authors determined that basal translation levels were increased in FMRP-deficient animals compared with those injected with a scrambled morpholino; strikingly, no changes in tectal neuropil translation were detected after training in FMRP-deficient animals. Furthermore, plasticity of the avoidance behavior after visual conditioning was disrupted in FMRP knockdown animals 24 h after training. Together, these data led the authors to conclude that translational control by FMRP is important for the maintenance of behavioral plasticity in *Xenopus*. Given the lack of other measures of experience-dependent alterations of local protein synthesis in intact preparations, the results in Liu and Cline (2016) are important to our understanding of the role of FMRP translational control in regulation of behavior. Moreover, the optimization of the spatiotemporal resolution of FUNCAT to assess region-specific translation could inspire subsequent studies in other animal models and brain areas to uncover FMRP function across evolution.

Although the conclusions of Liu and Cline (2016) are based on strong evidence and well-designed experiments, future studies using a similar experimental approach should consider two technical concerns related to measurements of local translation and the use of colchicine in an FMRP knockdown background. Deletion of FMRP can lead to an abnormal upregulation of the microtubule-associated protein 1B (MAP1B) providing more stability to microtubules (Lu et al., 2004). Therefore, a positive control demonstrating that colchicine effectively blocks microtubule polymerization when FMRP is knocked down would strengthen the experimental approach of Liu and Cline (2016). Furthermore, the mechanism of colchicine action remains somewhat puzzling. In the Liu and Cline (2016) study, colchicine application is meant to prevent protein trafficking along microtubules, but RNA-binding proteins, such as CPEB, traffic normally, although they presumably rely on the same transport mechanism. After colchicine treatment, one would expect to see a reduction in FUNCAT signal in the neuropil because the somatic

protein contribution is cut off (Liu and Cline, 2016, their Fig. 4d) and an increase in mitochondrial fluorescence intensity in the cell body layer of colchicine-treated animals as these organelles accumulate (Liu and Cline, 2016, their Fig. 3fg). One possible explanation for not observing these changes could be that autophagy and/or proteolysis in the soma is upregulated to prevent abnormal buildup of proteins and organelles.

Liu and Cline (2016) provide a thought-provoking model to explain how FMRP results in the loss behavioral plasticity. They proposed that FMRP normally negatively regulates the synthesis of a “Degradation protein.” When FMRP repression is relieved, this protein would be synthesized, and it would degrade the proteins necessary for plasticity maintenance. Expanding upon this idea, other studies have demonstrated that, in rats, performance in behavioral tasks improves following inhibition of protein synthesis during a specific time window (Levitan et al., 2016). This process may be conserved across species because *Drosophila* FMRP mutants show memory impairments that can be ameliorated by inhibiting protein synthesis (Bolduc et al., 2008). In future studies, the concept of a “Degradation protein” could be tested by inhibiting proteasomal degradation during the behavioral task and assessing whether this rescues the maintenance of plasticity in animals treated with morpholinos against FMRP.

In addition to the well-documented role of FMRP in translational control (Darnell et al., 2011), other less studied functions of FMRP could be important for the maintenance of *Xenopus* behavioral plasticity. For example, FMRP could directly regulate ion currents (Deng et al., 2013). Therefore, knocking down FMRP may have an impact on behavior through changes in neuronal excitability. Is neuronal excitability altered in the tectum FMRP-deficient animals? It would be important to test whether local protein synthesis or an alternative function is the primary mechanism by which FMRP contributes to behavioral plasticity maintenance.

Liu and Cline (2016) provide a novel approach to assess the complex landscape of protein synthesis with FUNCAT in intact brain areas following behavioral manipulations. Moreover, this paper demonstrates the importance of FMRP translational control in behavioral plasticity and raises a number of questions for future research. For example, do different learning and memory tasks recruit different sources of protein synthesis (i.e., local

vs somatic)? How do other translational control pathways, such as the mammalian target of rapamycin and the eukaryotic translation initiation factor 2 α pathway, interact with FMRP to regulate local protein synthesis? Is the role of FMRP in behavioral plasticity conserved across species? The transparency of *Xenopus laevis* allows *in vivo* newly synthesized protein measurements, but more innovation will be needed to develop techniques to measure local protein synthesis *in vivo* in other systems. Further research along these lines could be critical to the development of treatments for FXS. The cutting-edge study of Liu and Cline (2016) sets the stage to address these questions and to understand how local protein synthesis is regulated by experience to alter behavior, an extremely exciting prospect for the neuroscience field.

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