

# Intracellular distribution of CPEB protein Orb2 in brain neurons of *Drosophila melanogaster* depends on 3'UTR of Orb2-coding mRNA.

P-03.1-12

E. Kozlov<sup>I</sup>, M. Zhukova<sup>I</sup>, R. Gilmudtinov<sup>I</sup>, P. Schedl<sup>II</sup>, Y. Shidlovskii<sup>I,III</sup>

<sup>I</sup>Institute of Gene Biology RAS, Moscow, Russia, <sup>II</sup>Department of Molecular Biology, Princeton University, Princeton, United States of America, <sup>III</sup>I.M. Sechenov First Moscow State Medical University, Moscow, Russia

CPEB proteins take part in translation activation mediated by cytoplasmic polyadenylation. CPEB protein Orb2 from *D. melanogaster* is expressed in central nervous system of adult flies. Compromised Orb2 expression leads to impairment in learning and long-term memory formation. Presumably, 3'UTR of Orb2 mRNA participates in protein translation via autoregulatory processes. To study this mechanism, we have deleted the most part of 3'UTR of Orb2 gene bearing multiple CPE-sequences with help of CRISPR/Cas9 system, resulting allele was designated as Orb2R. Immunofluorescence staining of brain of Orb2R flies using antibodies against Orb2 protein displayed a drastic reduction of its level in neuron outgrowths compared to wild type, while Orb2 level in cell soma was the same. Synaptosome fraction prepared from Orb2R fly brain extracts contained 5 times lower Orb2 protein amount than wild type had, although total Orb2 protein level was 2 times lower than in wild type, probably due to decrease of mRNA stability. Obtained results allow us to suggest the following model of Orb2 expression regulation: Orb2 protein, being translated in cell body, binds its own mRNA and transfers it to synapses in complex with a transport protein. Earlier studies have shown the ability of Orb2 to facilitate dendritic transport of several mRNAs, Orb2 also can bind its own mRNA via CPE-sequences. 3'UTR deletion leads to decrease of Orb2 protein level only in synapses, that is why it is important for mRNA transport and on-site translation. In synapses translation from localized Orb2 mRNA starts, what causes predominant localization of Orb2 protein in synapses. This model is also supported with previously obtained data which have shown the prevalence of Orb2 protein localization in neurons outgrowths. Here we have shown the new role of autoregulation process in Orb2 expression depending on its intracellular localization. This work was supported by Russian Science Foundation grant 18-74-10051.