Role of STIM2 isoforms in the regulation of neuronal calcium channels in *Danio rerio*

Calcium ions play an important role in cell signaling. The cytosolic level of these ions increases as a result of cell activation and initiates intracellular processes leading to performing its function by a cell. Calcium ions enter the cytosol from the cellular stores called ER (endoplasmic reticulum) or from the external environment via calcium channels located in the plasma membrane. Refilling the ER in calcium ions takes place in the process of store-operated calcium entry. This process is based on the ability of sensory proteins located in the ER, called STIM, to sense too low calcium ER content and then to induce opening of calcium channels in the plasma membrane. ORAI and TRP are such channels. Regulation of calcium level is especially crucial for functioning of nervous system, since neurons excitability, i.e. neurotransmitters release, gene expression and morphological changes of dendritic spines depend on it. STIM2 dominates in brain but its functions, in contrary to STIM1, are less understood. In the literature, it is suggested that STIM1 may not only react to the changes in calcium level inside the ER, but it can also activate calcium influx in response to factors such as oxidative stress or temperature changes. Studies that would allow assessing if STIM2 possesses such features, and if so, which channel can be activated by it, have not been carried out yet. The aim of this project is to identify the channels interacting with STIM2 which, apart from regulating store-operated calcium entry, are involved in cellular respond to temperature changes or oxidative stress.

We are planning to carry out the research using zebrafish, which has two forms of this sensor called Stim2a and Stim2b. Using CRISPR/Cas9 technique we obtained fish lines devoid of either of them. During the first stage of the project, using *in situ* hybridization and immunohistochemical staining, we will localize Stim2a and Stim2b as well as Orai and selected Trp channels in zebrafish larvae and in adult fish brain slices. Simultaneously, we will investigate what is the impact of lack of each of Stim2 forms on the basic parameters of calcium homeostasis - spontaneous activity as well as response to stressors, like temperature increase or oxidative stress. Taking advantage of zebrafish transparency at the early developmental stages, we are going to record changes in calcium level *in vivo* in neurons of fish with GCaMP5G calcium probe expression in neuronal cells. We will also conduct calcium measurements in primary cultures from zebrafish neurons that will enable us to add information, which cannot be obtained from *in vivo* experiments.

To identify a channel that is interacting with Stim2 we plan to make an immunoprecipitation and to analyze bound proteins by mass spectrometry. The results of these analyzes will be confirmed e.g. by Western blotting and colocalization of Stim2 isoforms and their potential targets by the Proximity Ligation Assay. Interaction between calcium channels and Stim2a or Stim2b will be induced by treatment of zebrafish larvae or neuronal cultures with stressors like temperature increase or oxidative stress and by store-operated calcium entry induction. In order to determine phenotype resulting from lack of Stim2 we will analyze larvae mobility and reaction to light as well as behavior of adult fish in novel environment and in tests allowing the evaluation of cognitive abilities. By using RNA-Seq we will investigate level of various mRNA, in order to identify genes, which expression is changed in Stim2 mutants. In the final stage of the project we will attempt to restore the normal phenotype in cell cultures from Stim2 mutants via expression of missing proteins.

Calcium homeostasis is disrupted in a number of neurodegenerative states, including models of Alzheimer's, Parkinson's and Huntington's diseases analyzed by our group. Our studies and work of some other groups indicate an involvement of STIM2 in Alzheimer's disease pathology. Thus, understanding of function of Stim proteins and their role in regulation of calcium homeostasis in zebrafish will allow in the future the generation of models of different neurodegenerative disorders with altered calcium homeostasis.