Long-term space flight mediated changes in promoter landscape in Zebrafish (*Danio rerio)* tissues.

A.V. Cherkasov[1], K.V. Arshavsky[1], V.N. Sychev[2], M.A. Levinskikh[2], O.A. Gusev[1,3]

[1] *Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia*

[2] *Institute for Biomedical Problems, Russian Academy of Sciences, Moscow, Russia*

[3] *RIKEN, Yokohama, Japan*

Animal models are important to understanding influence of different factors of long-term space flights on living organisms and can be helpful for forecasting and prevention negative effects of space flights on humans.Teleost fishes as Japanese medaka (*Oryzias latipes*) and Zebrafish (*Danio rerio)* are one of the most popular model organisms in molecular genetics studies including space biology, primarily by sequenced and well-annotated genome, rapid external development, small size and relatively simple maintenance procedures aboard space-labs. Some previous experiments, which were performed on fish models using simulated microgravity and exposure aboard International Space Station (ISS), showed significant changes in fish behavior, physiology and whole genome gene expression. In this study we focused on changes in Zebrafish transcription initiation landscape, during long-term exposure in space environment. To define impact of space flight to tanscriptional activity on promoter level in Zebrafish tissues, we performed experiments using cap-analysis gene expression (CAGE) methodology. Usage of CAGE, instead more traditional RNA-seq approach, allows not only perform precise quantification of expressed transcripts, but directly map each transcription start site (TSS) with single nucleotide resolution to identify differences in promoter shape and TSS usage between “space” and “ground” conditions.

Two groups of Zebrafish individuals were used as experimental and control group. The individuals from experimental group were maintained in Aquatic Habitat (AQH) in ISS and fixated in RNA stabilization reagent immediately after arriving and after 36 days of staying aboard. Part of experimental group animals were returned alive from ISS for RNA fixation in ground conditions in two time-points: 2 and 36 days after return. The animals from control group were maintained in the same Aquatic Habitat system on the Ground with fully identical physical environment conditions and were fixated at the same time-points.

First results of CAGE shows the absence of significant differences in promoter shape, evaluated as the interquartile width of promoter area, during prolonged space-flight. However, we found significant impact of space flight conditions on transcriptional activity. More than 600 genes changed their expression in eye samples, after arriving aboard ISS. Notably, the number of

differentially expressed genes decreased to 154 after 36 days in space, it can be supposed successful adaptation to space flight conditions. Gene Ontology analysis of genes which overexpressed after two days aboard ISS shows significant over-representation of functional categories associated with circadian clock system, that confirm influence of microgravitation conditions to regulation of rhythmic processes in animals. Additionally, from GO categories enriched in genes up-regulated in all spaceflight time-points were found several closely related GO terms associated with transcriptional regulation. Among genes, characterized by this GO terms, four genes which are members of Activation protein 1 (AP-1): *fosb, fos, jdp2, junbb* show significant overexpression during spaceflight. This fact suggest deeply involvement AP-1 family members in organismal response to microgravity. Performed transcriptional analysis demonstrates both the considerable gene expression changes in zebrafish eye during long-term spaceflight and the successful adaptation to new environment.