Modeling of components of desiccation stress response in anhydrobiotic insect cell line

Alexander A. Nesmelov[1*], Ruslan M. Deviatiiarov[1], Elena I. Shagimardanova[1], Richard Cornette[2], Takahiro Kikawada[2]

[1]*Kazan Federal University, Kazan, Russia. *nesmelov@gmail.com*

[2]*Institute of Agrobiological Sciences, Tsukuba, Japan*

Oleg A. Gusev[1,3]

[3]*RIKEN, Yokohama, Japan. oleg.gusev@riken.jp*

A larva of an African chironomid *Polypedilum vanderplanki* is the most complex known organism able to enter specific ametabolic state - anhydrobiosis. This state enables larva to survive full body desiccation and resume life cycle after water return. Cell culture line Pv11, derived from the chironomid embryonic mass is the only currently available existing cell model for anhydrobiosis research. Anhydrobiosis in these cells, initially sensitive to desiccation, can be induced by continuous trehalose treatment. These cells able to survive desiccation outside the well-tuned protective environment of larvae interior due to an exquisite protective system, consisting of a large variety of proteins. Expression of these proteins becomes elevated on different stages of desiccation, revealing a presence of corresponding mechanisms of regulation. We hypothesized that regulation of expression of protective proteins in *Polypedilum vanderplanki* is performed by some ordinary system of stress response. Desiccation of *Polypedilum vanderplanki* cells causes a complex stress composed of osmotic and oxidative components. Osmotic stress caused by action of protective sugar trehalose, which is strongly accumulated in larva during desiccation and condensed cell milieu. Also, desiccation causes accumulation of reactive oxygen species. Since onset of dry season is also related to the elevation of temperature in *Polypedilum vanderplanki* habitats, we also considered heat-shock as a possible trigger of gene expression in response to desiccation.

In our experiment, Pv11 cells were exposed to a number of stress conditions, including osmotic stress, oxidative damage and heating. Osmotic stress was modelled by treatment with trehalose, mannitol and NaCl, oxidative damage was achieved via paraquat treatment. For

heat-shock, cells were heated at 42°C. Cells were sampled at different timepoints (1, 3, 24, 48 h) and processed for RNA sequencing using Illumina TruSeq kit (single end, 50 b.p.) and Illumina HiSeq 2500 platform. Reads were mapped to existing *Polypedilum vanderplanki* genome with gene models PvGeneModel_v0.91.gff (http://bertone.nises-f.affrc.go.jp/midgebase/) using TopHat 2.0.13 and HTSeq [1,2]. Differential expression analysis was performed with R packages DESeq и edgeR [3,4]. Overrepresented go terms were discovered using topGO [5].

On a large scale, most significant changes in gene expression were observed after treatment by trehalose, mannitol and NaCl. Samples after heat shock are grouped with control and cells treated by paraquat for ROS induction are most closely related to control in terms of gene expression. We observe also a clear difference between osmotic stresses and other treatments from the view of rate of gene expression change: ROS and heat-shock cause a rapid response within 3 h post-treatment, whereas osmotic stresses cause slow elevation of gene expression.

Previously we revealed a set of genes with proposed role in defense of cell components against desiccation damage. These genes ("anhydrobiotic genes") were identified via their elevated expression during water loss in the whole larva. Pattern of expression of these genes in Pv11 cells under different stress conditions is dissimilar to that previously observed in the larvae body . Genes with elevated expression after paraquat treatment include no single, "anhydrobiotic genes") moreover, ROS induction cause a decrease of expression of most of such genes. After heat shock treatment, six anhydrobiotic genes become activated including thioredoxins, methyltransferase, LEA (late embryogenesis abundant) protein. Interestingly, among a number of heat-shock proteins in anhydrobiotic genes group, heat-shock treatment activates only a single one.

Trehalose and NaCl treatment of Pv11 cells for 24 h causes an upregulation of a largest number of anhydrobiotic genes: 60 and 62, correspondingly. However, treatment of Pv11 cells with individual components of desiccation-related stress is unable to mimic transcriptome response identical to the desiccation of the whole larva. Remarkably, pattern of gene expression after ROS treatment is highly dissimilar to that during desiccation. This indicates that ROS-related system of gene regulation is unlikely to be involved in regulation

of genes in response to desiccation, despite a dramatic increase of ROS production during water loss.

This work was done in the frame of the Program of competitive growth of Kazan Federal University among world class academic centers and universities. mRNA sequencing and bioinformatics analysis was performed under support of grant by Russian Scientific Foundation for international groups 14-44-00022. Cell culturing and experiments were performed with support of grants by Japan Society for the Promotion of Science (JSPS) - 16K15073 and 15H05622

1. Alexa, J. Rahnenfuhrer (2016) topGO: Enrichment Analysis for Gene Ontology. R package version 2.28.0.
2. Simon, W. Huber (2012) Differential Expression of RNA-Seq Data at the Gene Level–the DESeq Package.
3. Simon, P.T. Pyl, W. Huber (2015) HTSeq-A Python Framework to Work with High-Throughput Sequencing Data. *Bioinformatics*, **31**(2):166–69.
4. M.D. Robinson, D.J. McCarthy, G.K. Smyth (2010) edgeR: A Bioconductor Package for Differential Expression Analysis of Digital Gene Expression Data. *Bioinformatics*, **26**(1) 139–140.
5. Trapnell, L. Pachter, S.L. Salzberg (2009) TopHat: Discovering Splice Junctions with RNA-Seq. *Bioinformatics*, **25**(9):1105–1111.