Perspectives of RNA/DNA studies using latent stages of invertebrates and plants exposed to space flight and outer space environments

Oleg Gusev[a, b], Takashi Okuda[c], Vladimir Sychev[d], Margarita Levinskikh[d] and Manabu Sugimoto[b].

[a] Kazan State University, Kazan, Kremlevskaya str., 18, 420008, Russia

[b] Okayama University, Tsushima, 3-1-1, Okayama, 700-8530, Japan

[c] National Institute of Agro-Biological Sciences, Tsukuba, 2-1-1, Kannondai, Ibaraki, 305-8602, Japan

[d] State Scientific Center of Russian Federation, Institute of Bio-medical Problems, Moscow, Khorosheevskoe shosse, 76a, 123007, Russia

E-Mail: oleg@cryptobio.com, ogusev@mail.ru

Abstract: We report preliminary data of analysis genomic DNA stability and gene expression both in active and dormant stages of number of invertebrates and a plant exposed to real space flight environment onboard ISS during 2005/2006. Perspectives of DNA studies using latent stages of living organisms exposed to outer space in the forthcoming space programs are discussed.

Key words; International Space Station, dormant stages of plans and invertebrates, hsp90, gene expression, genomic DNA stability.

Limitations related to the cargo space and water usage in the real space environment are the main factors influencing the planning of the biological experiments onboard spacecrafts. In the long-term space flight to other planets, one should consider using live animals as an important component of Life Support Systems (LSS), objects for biomonitoring and research procedures (Horneck, 1999). Many species (plants, and both invertebrates and vertebrates) were able to adapt to the microgravity and other factors of space flight onboard the spacecraft, but, in contrast to plants long-term cultivation (egg to egg) of active animals in space has not yet been conducted. An alternative way is to use latent stages, such as cysts, resting eggs or cryptobiotic stages of living organisms. The most attractive among these options are animals possessing an unhydrobiotic feature, being able to drastically decrease, or even stop metabolism in the absence of water, but stay alive for years (Watanabe, 2006). A number of cryptobiotic forms, representing different group of pro- and eukaryotes have already be proven to be fully tolerant to the space flight environment, and some prokaryotes and fungi have been confirmed to be able to resist outer space environment for long periods of time (Gaubin et al., 1990). Studies also suggest that, in many cases, the active forms of the organism revived from the cryptobiotic stage and then exposed to space environment shows changes in their viability, rate of metabolism, gene expression and other features of their life cycle (Clegg et al., 2000).

The outer space environment represents a combination of extreme factors, such a space radiation, space vacuum, microgravity, and temperature fluctuations. In the next few years, several independent programs are scheduled to testify the tolerance of multi-cellular organisms to such harmful conditions (ESA "EXPOSE" and "Biopan", Russian "BIORISK" projects). The effect of long exposure to the outer space environment on the RNA and DNA stability in the dormant stages of higher animals and plants is one of the particularly interesting subjects to investigate.

Both tardigrades (water bears) and branchiopod crustaceans (brine shrimps and triopses) are included in the list of species for the forthcoming experiments of the exposure of dormant stages of prokatyotic and higher eukaryotic (for the first time in the world) organisms to the outer space environment for the long period of time ("Biopan", "EXPOSE" and "Biorisk" research programs). Those experiments would provide a unique physiological and biochemical data of the resistance of the dormant stages of the multicellular organisms of different level of organization the complex of the extreme factors, such as space vacuum, microgravity, space radiation and temperature fluctuations in the outer space. DNA and RNA stability and expression data would be one of the major sources of information about influence of the outer space on the biological object and organization of the post-flight genetic experiments is matter of high priority in the planning of such projects. Considering the cargo limitations of space for model organisms in these planned outer space experiments, we present here preliminary data of the genomic DNA extraction efficiency from the dormant and active stages of tardigrades and branchiopod crustaceans. In addition, we report the first results of monitoring the stress response ability changes in *Artemia salina* larvae revived from the encysted embryos and incubated for period 1 and 8 month under real space flight conditions onboard the Russian segment of International Space Station (ISS).

Our experiments showed that both dormant and active stages of water bears and branchiopod crustaceans are quite capable for post-spaceflight DNA analysis. However, the yield and size of the extractable molecules of DNA is different between active and dormant animals subjected to the same DNA extraction procedure. We suppose that lower yield and less fragmented structure of the DNA in the case of dormant stages of all tested invertebrates is a result of the presence molecular chaperones and other stabilizing agents, such as trehalose in tuns and encysted embryos. Being involved in the formation of the "glacial package" around organelles of the dried cells, these agents would prevent DNA molecules from fragmentation during intensive homogenization with ceramics beans, but at the same time, excess of the saccharides and other preservative agents in the dormant states should have decreased the total yield of DNA during the purification procedures.

The data on the activated larvae of crustaceans (which is 60-80 ng per specimen) is still preliminary and should be recalculated according to the stage of active animals to be used in concrete experiment, because of the rapid increase of cells number after hatching through the serial larvae stages (Suzuki, 2003). At the same time, data on the tuns and active stages of tardigrades can be considered conclusive (30-40 pg per tun). We have used adult water bears, so that number of cell, and, correspondingly, average amount of DNA would not be subject to change after reactivation in a water environment. Our data indicate that both groups of invertebrates (active and dormant) are quite suitable for DNA analysis, including methods which require high size of DNA molecules. Moreover, in the case of branchiopod crustaceans, PCR analysis would be possible using genomic DNA extracted from the single encysted embryos (10-12 ng of DNA extractable from a single egg).

We found that exposure to the space environment depressed the ability of larvae of *Artemia* to resist the heat stress, but it is important to be careful with these conclusions (Gusev et al, 2006). The range of the temperatures differences in the expression of the hsp90 mRNA was found to be different between space flight and control groups (36-40°C) represent extreme thermal conditions for the larvae, since the average temperature in natural environment lays in the limits of 20-26°C.

Further experiments, which are now quite possible on the basis of these programs, including the global analysis of RNA expression in the dormant stages of these

animals being activated in space, are needed to further understand the complex molecular adaptations of the invertebrates to the long-term space flights.

In autumn of 2006 unique experiment of growing barley from seeds under real space flight environment was conducted onboard ISS in "LADA". A month old sprouts were transported back to Earth and immediately frozen. Preliminary experiments showed that RNA and DNA in the tissues remained stable and at the moment we are conducting of molecular analysis of grown in space barley plants compared with ground control, aiming to clarify stress genes expression patterns.

Clegg, J.S., Jackson, S.A., Van Hoa, N., Sorgeloos, P. Thermal resistance, developmental rate and heat shock proteins in *Artemia franciscana*, from San Francisco Bay and southern Vietnam. J. of Exp. Mar. Biol. Ecol. **252**, 85-96, 2000.

Gaubin, Y., Delpoux, M., Pianezzi, B., Gasset, G., Heilmann, C., Planel, H. Investigations of the effects of cosmic rays on *Artemia* cysts and tobacco seeds; results of Exobloc II experiment, flown aboard Biocosmos 1887. Int. J. Rad. Appl. Instr. **17**, 133-143, 1990.

Gusev O., Ziegler, T., Saigusa, M. Expression and structure of stress chaperon hsp90 in terrestrial decapods, *Coenobita* (*Anomura*: *Coenobitidae*) and *Chiromantes* (*Brachyura*: *Sesarmidae*). Crus. Res., **6**, 109-119, 2006.

Horneck, G. European activities in exobiology in Earth orbit: Results and perspectives. Adv. Space Res. **23**, 381-386, 1999.

Suzuki, A.C. Life history of *Milnesium tardigradum Doyere* (tardigrada) under a rearing environment. Zool. Sci. **20**, 49-57, 2003.

Watanabe, M. Anhydrobiosis in invertebrates. Appl. Entomol. Zool. **41**, 15-31, 2006.