Gene expression variations during *Drosophila* metamorphosis in real and simulated gravity

R. Marco (1), L. J. Leandro-García(1) , Alberto Benguría (2), R. Herranz (1), A. Zeballos (2), G. Gasset (3), J. J. van Loon (4) & F. J. Medina (2)

(1) Dept. Bioquím. IIB, UAM-CSIC, Madrid. Spain (Phone: 34-914975409, roberto.marco@uam.es). (2) CNB, CSIC, Madrid. Spain. (3) GSBSM, U. P. Sabatier, Toulouse, France. (4) DUSC, Amsterdam, NL (5) CIB, CSIC, Madrid, Spain.

Establishing the extent and significance of the effects of the exposure to microgravity of complex living organisms is a critical piece of information if the long-term exploration of near-by planets involving human beings is going to take place in the Future. As a first step in this direction, we have started to look into the patterns of gene expression during *Drosophila* development in real and simulated microgravity using microarray analysis of mRNA isolated from samples exposed to different environmental conditions. In these experiments, we used Affymetrix chips (version 1.0), containing probes for more than 14,000 genes, almost the complete *Drosophila* genome, 55% of which are tagged with some molecular or functional designation while 45% are still waiting to be identified in functional terms. The real microgravity exposure was imposed on the samples during the crew exchanging Soyuz 8 Mission to the ISS, in October 2003, when after 11 days in Microgravity, the Spanish-born astronaut Pedro Duque returned in the Soyuz 7 capsule carrying the experiments prepared by our Team. Due to the constraints in the current ISS experiments in these Missions, we limited the stages explored in our experiment to the developmental processes occurring during Drosophila metamorphosis. As the experimental conditions at the launch site, Baikonour, were fairly limited, we prepared the experiment in Madrid/Toulouse and transpòrted the samples at 15^{o}C in a temperature controlled container to slow down the developmental process, a temperature previously established to be fully compatible with normal pupal development. The samples were transfered to room temperature just before hand-over 12 hours before launch, and immediately upon arrival into the ISS were inserted in an incubator at 25^{o}C. Fixation took place in the ISS using an automatic modification of the Berlingot concept developed in Toulouse, almost four days after the pupae were transferred to room/25^{o} temperature After recovey in Moscow, pupae were homogenized and the mRNA used to detect the level of gene expression using the Affymetric chips. In parallel, a set of controls were run under the same conditions but the exposure to the Space Station environment (Microgravity) as well additional elements of the launch and landing process. A similar group of pupae were exposed to simulated Microgravity in the RPM (DUSC, Amsterdam). In this case, two sets of samples were used, one incorporating a cold transport step equivalent to the one used in the Space experiment while a second was maintained all the time at

normal temperature, without this transport step. In the group maintained all the time at normal temperature, a third group of samples were treated during the same period of time in the Medicar Centrifuge at 10g. The analysis of the experiments still in progress show several interesting features: a. a significant group of genes, of the order of 10% of the whole Drosophila complement, show a significant and modified response to real microgravity (ISS). Most of them are similarly affected in the RPM (simulated Microgravity). Only very few, less than 0,5% differ in response in real and simulated Microgravity. Although a significant group of the affected genes in the RPM are also modified in the experiment run all the time at normal temperature, simply exposure to a storage at 15^{o}C during five days before pupal development also modifies the expression level of many of these genes even though the subsequent incubation during almost 4 days is done at normal temperature. A more restricted group of genes was affected after being exposed to 10g centrifugation. Several important conclusions can be drawn from these data: a. Microgravity, both real and simulated, produces a significant effect on gene expression of a relatively large group of genes, b. the RPM seems to be a good instrument to run simulations of the Space Microgravity conditions on the ground and c. gravity can act synergically with other environmental cues to induce important changes in the gene expression patterns during *Drosophila* development, a conclusion that supports the importance of maintaining an active Research Program on the long-term response of living organisms to the environmental conditions that will be found altered outside the Earth in the next phase of Space exploration.