The "Gene" Experiment in the Spanish Soyuz Mission to the ISS. Effects of the cold transportation step

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Abstract

If exploration of outer space is going to be a major human enterprise in the future, it is important to establish the nature of the biological response to the space environment. In one of the recent Soyuz missions to serve the ISS, the Spanish Soyuz Mission in October 2003, we sent a group of Drosophila pupae that underwent almost complete development there. Microarray analyses of the RNAs extracted from flies fixed in the ISS revealed that a relatively large set of genes (15% of the total number assayed) suffered a significant expression change in these conditions. Furthermore, the samples had to be transported to the launch site and it was necessary to slow down their development by exposing them to a lower temperature, fully compatible with pupal development. Such a pre-exposure had an effect by itself on the pattern of gene expression observed after pupal development at normal temperature, but the two environmental factors seemed to act synergistically. These findings indicate the importance of maintaining a vigorous scientific program in the ISS to understand the consequences of the modified environment in outer space on living organisms.

1 Introduction

In the adventure of exploration/colonization of outer space, many biological beings are going to be at least part of life support systems in long-term human bases in near-by planets. On the other hand, it is clear that the living systems are well adapted to the different environmental conditions existing on the Earth. Gravity, although weak, is a constant force acting on all objects on the biosphere and, thus, it is necessary that all living organisms are well adapted to its presence. The case of the plants is paradigmatic, but what about animals? The availability of research opportunities in space has allowed to find out that even microscopic systems are able to sense and respond to the modification in this parameter (reviewed in 1). Paradoxically, large multicellular organisms have been more robust and seem to be able to withstand exposure to strange environments such as those present in the different flying ships in which they have been sent into space. Although some transient modifications have been reported, especially in the case of amphibian development, systems covering almost all the phylogenetic tree have been flown and normal development has been the general outcome of these experiments (2). The International Space Station is the current flight facility to perform microgravity experiments for the new phase of space exploration and colonization.

2 The experiment background

Drosophila melanogaster is one of the main model systems in current biology. Most of what we know about how animal development occurs has been derived from pioneer research using this organism. The whole sequence of its euchromatic genome was obtained in 2000 (3). The genome sequence completion has made possible that chips incorporating probes to test the expression of a large amount of genes became commercially available for *Drosophila* researchers almost immediately. Thus, we decided to take advantage of this fact and conceived our "*Gene*" experiment. The experiment intended to study the effects of the space environment on the gene expression pattern of *Drosophila melanogaster* pupae exposed to microgravity during their development. In doing so, we could rely on previous experiments performed in our laboratory to adapt fixation methods to the space conditions (4). Nevertheless, because we could not use the only glovebox that had been previously installed in the ISS, the experiment had to be run inside an ESA type I container to meet the level of containment imposed by the safety measures for toxic fixatives in space. Even the original berlingot concept (5) had to be adapted to the lack of a glovebox. The concept involves putting the samples inside oxygen permeable double plastic bags while the fixative remains inside glass ampoules until the ampoules are broken. Dutch Space had quite recently developed a keen modification of the concept, the MAMBA hardware, "Motorized Ampoule Breaker Assembly". In a meeting in January 2003 when our experiment was being discussed with the ESA personnel in ESTEC, Dutch Space representatives introduced to us this type of hardware and offered us to use their flight qualified units (see a picture of the MAMBA in 6). Nevertheless, an important problem remained to be solved: the availability of a power supply to activate the MAMBA containers in the ISS. After some inquires with representatives of different European space industries including Spanish ones, we decided to accept the offer of Dutch Space to produce such power supply. While these inquiries were advancing, the news of the Columbia accident modified the prospects of our flight. It was clear soon that the Spanish Soyuz Flight was not going to be launched in April but would be delayed. The power supply was ready in

June 2003, so that it was launched in the August Progress 12P cargo flight to the ISS, in time for the Spanish Soyuz Mission in October, 2003.

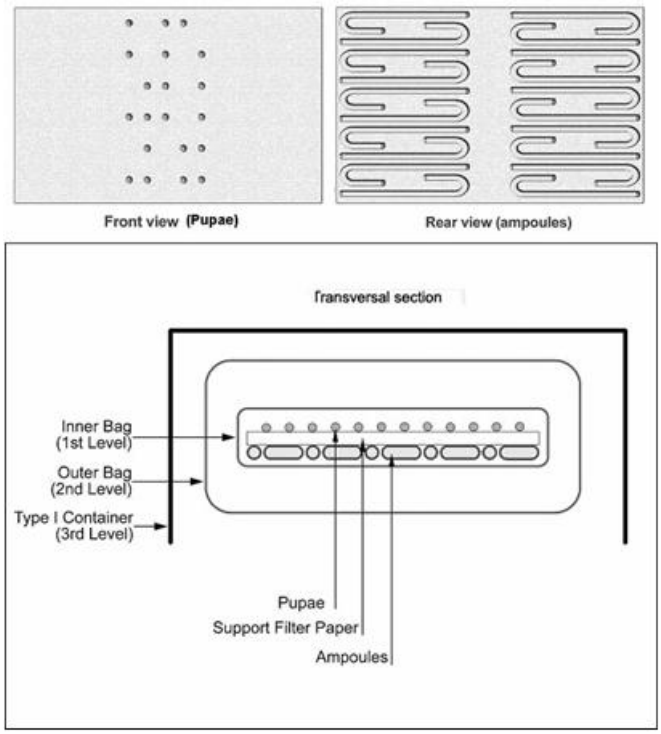

Fig. 1. Berlingot concept adaptation to the Gene experiment.

3 The Gene experiment

Twenty *Drosophila melanogaster* Oregon R wild type larvae/early pupae (Fig. 1) were transferred to the centre of a filter paper of the appropriate size to fit in the double bag (3.2 x 10 cm). The larvae were already migrating out of the food and starting pupation but were still easily detached from the plastic wall of the tube and transferred to the filter paper. The glass ampoules were filled with the fixative under low pressure as previously described (5). As many ampoules as possible were laid inside the berlingot. Since each ampoule held 30 µl and 20 ampoules were inserted per berlingot bag, 0.6 ml of acetone was released on the pupae when the ampoules were broken.

Due to these constraints, we proposed to limit our experiment to a particular developmental process, namely, *Drosophila melanogaster* pupation. The whole pupation process requires four days at normal room temperature, 22ºC. During this time, the animals remain immobile inside the pupal case. They do not require any food, but only oxygen. A second problem arose when we realized that the berlingot preparation process was relatively complicated and required the use of dedicated equipment available in Toulouse, but not easy to be transported to the launch site in Baikonour. We had previously been testing the capability of *Drosophila* to survive at relatively lower temperatures (7). They develop normally but more slowly. Thus, we came to the conclusion that if we could transport the MAMBAs with the berlingots and the pupae prepared in Toulouse at 14ºC, we could hand over our experiment the previous night before launch and be able to expose our pupae for three and half days to microgravity at normal temperature. ESA provided one of the thermostatized transport containers built by COMAT in France capable of maintaining a fixed temperature during relative long periods of time.

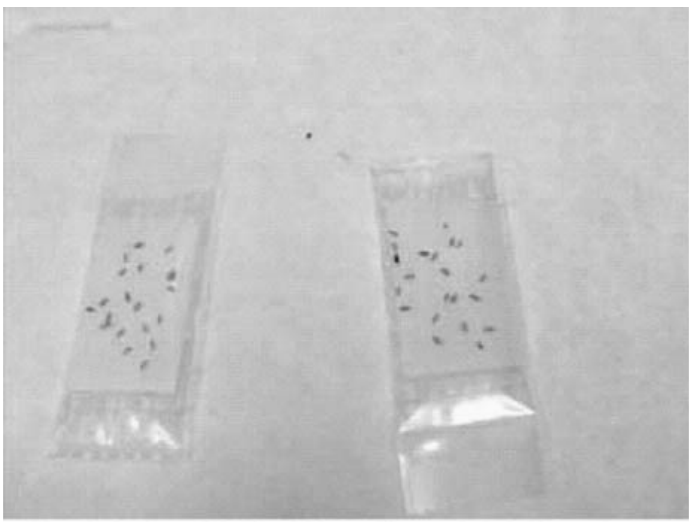

Fig. 2. The Drosophila pupae from the ISS experiment after recovery in Moscow.

4 Meeting the additional constraints of the experiment

As already mentioned, when the Gene experiment for the Spanish Soyuz Mission was decided, we had already succeeded in adapting several of the methods for *Drosophila* fixation to be used in space. For RNA and protein extraction, the simpler and optimal method has been to expose the animals to dehydrated acetone to remove as much water as possible from the tissues. Acetone dehydration will work through the thick cuticles of the pupae and imagoes. It is a method easy to be adapted to the berlingot concept as explained above. Once the acetone is released exposure of the samples to low temperatures increases the recovery of the RNAs. Therefore, this is what was accomplished in the ISS by Pedro Duque. The results shown in Table I indicate the acceptable quality of the RNA samples for analysing the gene expression changes with microarray technology. The RNA extraction method used Trizol for homogenization and posterior isolation (4).

Experimental condition	*Microgravity on I.S.S.*		*1g control (for ISS)*		*1 g control without cold*	
Sample Name	FG3	FG4	GG4	GG5	91	92
Scale factor	2.163	2.028	1.560	1.543	2.709	2.784
Present Probe sets	6041	6406	7231	7470	4989	5493
Present %	43.1%	45.7%	51.6%	53.3%	35.6%	39.2%
Average signal (Present)	811.3	758.7	615.7	569.1	926.9	893.6
3´/5´ Ratio (Actin)	2.71	4.84	1.97	1.79	2.68	2.01
3´/5´ Ratio (GAPDH)	2.18	3.09	2.02	2.44	2.31	2.01

Table I. Some reference values that indicate the quality of the RNA samples for the microarrays analysis.

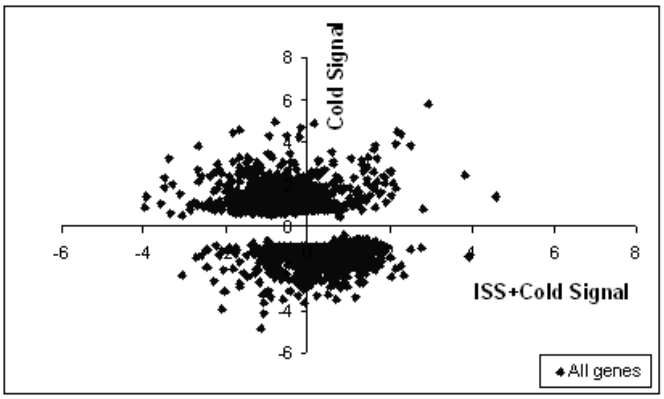

Figure 3A: Response of the genes in the ISS. In the abscissa, the changes (logarithmic scale) in the microarrays gene expression levels of the space flown samples are plotted compared to the cold transported controls. In ordinates, the expression levels of the cold transport controls compared to the controls at normal room temperature are shown. The genes that do not change significantly upon cold transportation do not appear (empty region in the figure).

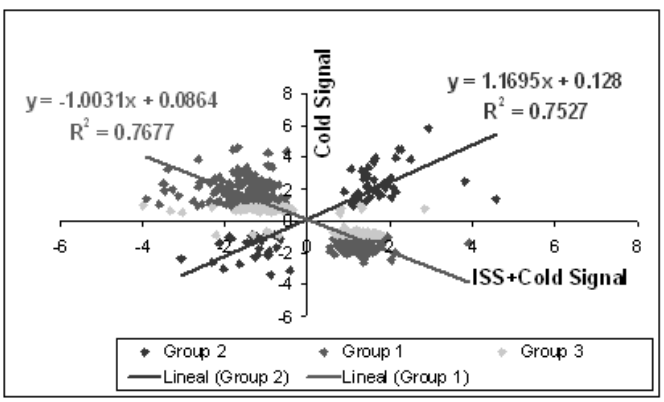

Figure 3B: Groups of genes significantly responding to the ISS conditions. Group 1. Genes that counteract in space the effect of cold transportation (2d and 4th quadrants): 327 genes (dark green diamonds). The line is the regression line through these values. Group 2. Genes that change in the same direction under both conditions, namely, further increasing or decreasing in microgravity (1st and 3d quadrants): 77 genes (red squares). The line is the regression line through these values. Group 3. Genes that do not change significantly by the cold transport and are significantly affected in microgravity: 894 genes (cyan squares). They can be assimilated to groups 1 and 2, depending whether they are in the 2rd and 4th quadrant (361+518 genes) or in the 1st and 3 rd quadrant (7+8 genes).

5 The experiment outline

The actual time line of the experiment was the following. The two Gene containers with the rest of the additional Aging and Root containers were inserted in the Biology Transport Container, delivered in Baikonour and launched in the Soyuz 7 flight to the ISS on October 18, 2003 at 11.37 local time (5.37 GMT). After docking the containers were transferred to the ISS, where they were inserted in a holder and installed in the Aquarius 2 incubator in the Russian segment at 22ºC. On the 21st, at 15.46 GMT, the two Gene containers were removed by Pedro Duque and inserted in the power supply that activated acetone release from the ampoules. At 15.50, the two containers were introduced in the cryogen freezer at -22ºC in the Russian segment of the ISS. They remained there until Monday 27th, when at 17.45 GMT they were brought into the Biology Transfer Box and transferred to the Soyuz 6 capsule that returned to the ground with Pedro Duque and the previous crew in the ISS. Landing took place on October 28 at 2.45 GMT and the containers were immediately transferred back to a transportation box at 3ºC to be taken back to Moscow where, at Star City, they were delivered to the scientific team around 16.09 GMT. After moving them to the provisional laboratory set by us there, the containers were open, the pupae removed from the plastic bags and homogenized in Trizol less than an hour later. The RNAs in the pellets were put on dry ice where they remained until we could flight back to Spain. An almost parallel experiment, using pupae prepared a day later and exposed to 14ºC during a similar period of time was performed. After the successful launch, the samples were removed from the low temperature and the ampoules were broken after a similar incubation at 22ºC. A similar group of pupae not subjected to this cold transportation step was also fixed after an equivalent time of development and its RNA extracted in the same way.

6 Results of the Gene Experiment

In Figure 3 a representation of genes modified in space *vs* the changes produced merely by the cold transport itself is shown. When compared to the parallel controls which had undergone the same treatment but the space flight, it turned out that a relatively large amount of genes, more than a thousand, had significant expression level alterations (Blue diamonds in Fig. 3A). Furthermore, when the gene expression patterns in the controls that had been exposed to a similar period of exposure to 14ºC as the flight samples (parallel controls) were

compared to pupae from the same flies not exposed to this cold step, many genes were also modified by such a treatment. It is interesting to note that both treatments (exposure to 14ºC or to the space conditions) had been previously found compatible with a normal development of flies that kept successfully breeding after these treatments. It turned out that the two treatments showed some kind of synergism. In fact, the genes modified by the exposure to the space conditions actually fall into three categories. A) Group 1 of genes (317) that had been previously modified by the cold treatment and that in microgravity were returning to the normal conditions much faster than the samples not exposed to this environmental change (dark green diamonds in Fig. 3B). B) Group 2 of genes (77) that were modified by the microgravity treatment even more than by the cold treatment alone, *i. e.*, they increased/decreased even more than in the parallel controls (red squares in Fig. 2B). The regression line has a slope of 1.17, indicating that the effect of microgravity was stronger than that of the cold treatment by itself. C) Group 3 of genes (894) not significantly modified by the cold treatment that were changing in microgravity. They can be assimilated to the other two groups since most of them (879) change in opposite direction in both treatments and only 15 (similar to group 2) change in the same direction. There are reasons to believe that the synergism between the two treatments is responsible of the changes in group 2 and possibly also in part those in group 3 (the underlying reasons will be analyzed elsewhere, Marco et al, in preparation). In future work, we will analyze how many of the genes affected in space are modified in similar stressful conditions. Finally, we had carried a similar comparison with the genes modified in ICE First, the *Caenorhabditis elegans* experiment run in the DELTA mission (8).

7 The continuation of the analysis

It is not simple to perform experiments in space. Until a new flight opportunity becomes available to us, we are limited to perform experiments in the Ground Simulation Facilities available in our laboratories. This is what we are continuing doing. The results (8) are quite interesting. So far, we have performed similar experiments in the Random Position Machine. The results show that as it has been the case in simpler systems (10 and this volume), the pupae developed in the RPM show a modification of a similar set of genes than in the ISS "Gene" experiment. Moreover, it has been possible to run there an experiment in the absence of a cold transportation step. By doing so, we have begun to identify that there is a particular set of genes that is affected by the absence of gravity (8). Magnetic levitation provides a different approach on the ground to microgravity. Experiments using this instrumentation equivalent to the Gene experiment are in progress. In particular, we are intrigued by the amplification of the expression changes by these treatments when additional modifications in the growing conditions are introduced, such as extreme temperatures, weak hypomorphic mutants that by themselves may not result in a strong enough phenotype, etc.

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