GENE EXPRESSION VARIATIONS DURING *DROSOPHILA* METAMORPHOSIS IN SPACE. THE GENE EXPERIMENT IN THE SPANISH CERVANTES MISSION TO THE ISS

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ABSTRACT

The ISS expedition 8, a 10 days "taxi" flight Soyuz Mission to the International Space Station (ISS) to replace the two-member ISS crew, took place during October 2003. Within the Spanish Cervantes Scientific Mission, in this crew exchanging flight, some biological experiments were performed. The third member of the expedition, the Spanish born ESA astronaut Pedro Duque, returned with the Soyuz 7 capsule and the experiment containing transport box after 11 days on microgravity. In the GENE experiment, we intended to determine how microgravity affects the organism rebuilding processes that occurs during *Drosophila* metamorphosis. In addition to the ISS samples, some control experiments were performed including a 1g Ground control parallel to the ISS flight samples, a Random Position Machine microgravity simulated control and a parallel Hypergravity (10g) exposed samples experiment. We have used extracted RNA from these samples to test the differences among gene expression during *Drosophila* development with one of the current more powerful technology, a *Drosophila* complete genome microarray (version 1.0, Affymetrix™). A preliminary analysis of the results indicates that around five hundred genes change their expression profiles being especially affected the mitochondrial ribosomal ones.

1. INTRODUCTION

One of the most challenging aspects of actual biology are the analysis of the complete genomes information. *Drosophila* genome has been sequenced [1] and powerful techniques like complete genome microarrays has become available to the scientific community. Taking advance of this technology (Affymetrix™ microarrays) and the possibility of implement a relatively simple experiment on a TAXI flight mission (Soyuz 8th mission to the ISS), we have studied the effect of three days of microgravity environment on ISS during three days, precisely during the pupation of some fruit flies.

Our group has been working in this field during several years. One of the first observation of us was the increase in motility, ageing and mitochondrial activity of flies exposed to microgravity in satellites missions [2]. After that results, the porpous of our group was to design a unit able to maintain a permanent colony of *Drosophila* in the ISS [3] and although that unit has become very useful for this purpouse we have no chance to test its in real Microgravity conditions. The results of an ESA topical team on Sample fixation [4], of which we are members, have been very useful in the conception of this experiment.

A preliminary analysis of the results have been presented below and briefly discussed until we complete the analysis of some complementary experiments that would greatly improve the statistical value of our results.

2. MATERIALS AND METHODS

2.1 Experimental system.

Dedicated preservation and fixation techniques were developed previously to this mission and finally tested in the International Space Station conditions. *Drosophila* larvae were selected just before the pupation start and assembled on a paper strip that was introduced in a berlingot containing ampoules filled with Acetone that was used as fixative. These berlingots were introduced in MAMBA's (Motorized Ampoule Breaker Assembly) and conserved at 14ºC until launch. Pupae developed in the Soyuz and the ISS during three days at 22ºC and then fixed with Acetone and conserved at nominal -20ºC. A theoretical similar experiment was developed paralelly on ground (1g control).

2.3 Microgravity simulation experiment

Using the Amsterdam USOC facilities we performed a experiment trying to reproduce exactly the ISS experiment conditions in a Randon Position Machine (putative microgravity) including a 1g control.

2.3 RNA extraction and microarrays technology

RNA was extracted from samples using a TRIZOL© commercial protocol and cleanup using RNAeasy Mini kit (Quiagen). RNA were processed as stablished for Drosophila genome microarray version 1.0 of Affymetrix™ (Ampliar por Alberto?).

2.4 Statistical analysis

To determine the number of up- or down-regulated genes in the microgravity conditions we used the affymetrix software tests (α_1=0.04, α_2=0.06, τ=0.015).

Experimental condition	*Microgravity on I.S.S.*		*1g control (for ISS)*		*Microgravity on RPM*		*1g Control (for RPM)*	
Sample Name	FG3	FG4	GG4	GG5	5	7	81	82

Scale factor	2.163	2.028	1.560	1.543	***2.709***	***2.784***	1.996	1.531
Present Probe sets	6041	6406	7231	7470	4989	5493	6997	7540
Present %	43,1%	45,7%	51,6%	53,3%	***35,6%***	***39,2%***	49,9%	53,8%
Average signal (Present)	811.3	758.7	615.7	569.1	926.9	893.6	622.5	560.3
3´/5´ Ratio (Actin)	2.71	***4.84***	1.97	1.79	2.68	2.01	1.93	1.97
3´/5´ Ratio (GAPDH)	2.18	3.09	2.02	2.44	2.31	2.01	2.33	2.11

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

Four comparisons were available for each pair of conditions (2 microarrays for each condition), so we take in consideration changes that occurs in the all of them. In a refined count, only the "present" probesets in the experimental condition and a signal log ratio ≥±1.0 were considered.

3. RESULTS

Due to the limitations of Space Research only two replicates of each experimental condition have been finally used in the microarray analysis. Some microarray general parameters as the scale factor or the number of present (detected from the background signal) probe sets (Table I) indicate the quality of the samples, but can also indicate a general reduction in the number of expressed genes (reflected in the reduction of the present probesets but the increase of the Average signal of them). A more rigorous parameter is reflected in the 3´/5´ ratios that should be between 2 and 3 but in the case of FG4 (ISS sample) is anomalous higher. Nevertheless the data seems to be valuable enough to be analyzed if we consider that is almost impossible to obtain this samples again.

In fig. 1 is represented the number of genes with a statistical significant change on the four analyzed conditions. From the analyzed 14010 probesets, around two thousand show consistent variations (changes in the four crossed comparisons) in microgravity conditions, of which 2/3 show a decrease in their expression. Being even more rigorous counting only changes with a Signal log ratio ≥±1.0, 275 probesets are increased in both ISS and RPM conditions and 252 are decreased. It must be added that the 90-95% of the probesets that changes only in ISS or RPM condition are also changed in the other condition although their change is not so robust to be detected by the severe statistical constrain of the microarray analysis. Only 6 probesets shown an increase on ISS conditions and decrease on RPM conditions, representing four ecdysone-induced gene 71 isoforms and two peptidases encoded by CG3355 and Jon65Ai genes.

Gene Ontology classifications allows us to provide also a first analysis of the biological processes involved in the response to Space environment (ISS) or just to microgravity simulation (RPM), although only half of the probesets included in the arrays represent genes with known GO properties. As reflected in fig.1 genes involved in mitochondrial ribosomes or electronic respiratory chain are robustly increased in the microgravity ambient. Muscle contraction and light response genes are also increased, as well as some reproduction related genes (these last group especially in the RPM microgravity simulation).

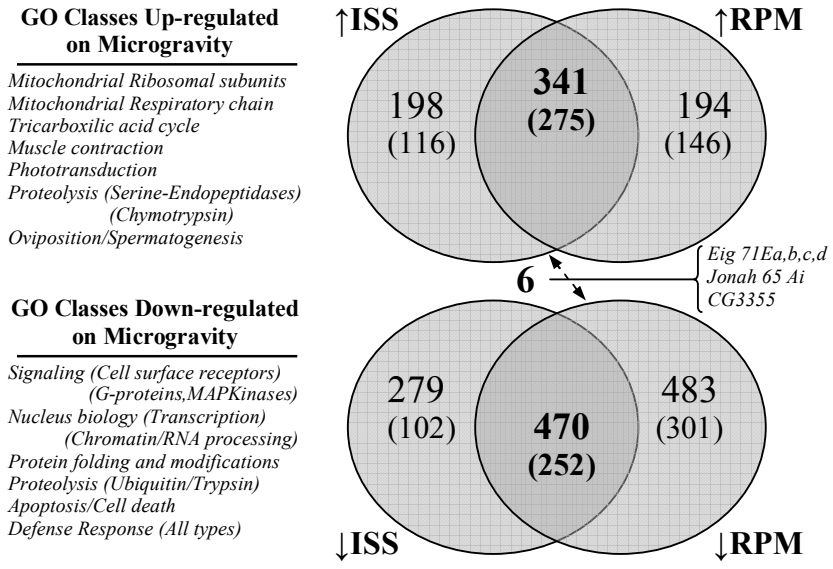

Fig. 1. Distribution of the up-regulated and down-regulated genes in microgravity conditions. Venn diagrams are used to indicate the number of probesets that increase their expression in ISS, RPM or both samples (top diagrams), or that shown a decreased expression on the sae conditions (bottom diagrams). 6 probesets are increased in ISS conditions and decreased in RPM microgravity simulation. At the left are indicated the biomolecular processes, as described by the Gene Ontology (GO) classification, that are more affected in microgravity.

	Gene Title	GO Cellular Component	Increased in ISS	Increased in RPM	Probeset present in ISS +	Probeset present in ISS -	Probeset present in RPM +	Probeset present in RPM -	SLR ISS	SLR RPM	average Fold
1	CG5023	Calponin-like	4	4	2	2	2	2	2,27±1,16	3,42±0,85	7,38
2	TpnC25D (II)	troponin complex	4	4	2	2	2	2	2,39±0,78	2,46±0,52	5,99
3	TpnC47D (Ib)	troponin complex	4	4	2	2	2	2	2,31±0,76	2,46±0,50	5,72
4	TpnC41F (IIIb)	troponin complex	4	4	2	2	2	2	1,94±0,57	2,42±0,23	4,54
5	Upheld (TpnT)	troponin complex	3	2	0	1	1	0	2,29±1,59	2,07±0,43	4,26
6	Paramyosin	muscle thick filament	3	3	2	2	2	2	1,48±1,14	1,56±1,15	3,81
7	flightin	muscle thick filament	4	4	2	2	2	2	1,85±0,71	2,11±0,07	3,78
8	wings upA (TpnI)	troponin complex	4	4	2	2	2	2	1,75±0,75	1,90±0,32	3,68
9	Upheld (TpnT)	troponin complex	4	4	2	2	2	2	1,69±0,84	1,86±0,33	3,49
10	Tropomyosin 2	muscle thin filament	4	4	2	2	2	2	1,69±0,78	1,80±0,72	3,45
11	TpnC73F (Ia)	troponin complex	4	4	2	2	2	2	1,59±0,60	1,88±0,55	3,44
12	Myosin (Mlc 1)	muscle myosin	4	4	2	2	2	2	1,57±0,64	1,69±0,28	3,42
13	Muscle LIM p. 84B	nucleus / cytoplasm	4	4	2	2	2	2	1,73±0,81	2,11±0,68	3,38
14	Muscle prot.20	muscle fiber	4	4	2	2	2	2	1,27±0,41	2,25±0,58	3,34
15	Zormin	cytoskeleton	0	0	0	0	0	0	0,35±0,10	1,89±1,54	3,32
16	TpnC41C (IIIa)	troponin complex	4	4	2	2	2	2	2,07±0,47	2,05±0,17	3,28
17	Tropomyosin 1	muscle thin filament	4	3	2	2	2	2	1,89±0,93	1,35±1,01	3,19
18	Myosin (Mhc)	muscle thick filament	4	4	2	2	2	2	1,53±0,76	1,29±0,61	2,81
19	Actinin	focal adhesion	4	3	2	2	2	2	1,30±0,79	1,38±1,06	2,75
20	Zormin	cytoskeleton	2	2	2	2	1	2	0,75±0,80	0,92±0,98	2,44
21	Actin 79B	muscle thin filament	4	2	2	2	2	2	1,18±0,71	1,02±0,80	2,25
22	Rya-r44F	integral to membrane	4	2	2	2	2	2	1,36±0,41	0,73±0,57	2,17
23	Actin 88F	muscle thin filament	4	3	2	2	2	2	1,19±0,50	0,99±0,42	2,16
24	cheerio	ring canal outer rim	3	4	2	2	2	2	0,89±0,41	1,17±0,44	2,11
25	Actin 87E	muscle thin filament	4	2	2	2	2	2	1,05±0,57	0,98±0,80	2,05
26	GST S1	muscle fiber transferase	4	4	2	2	2	2	1,21±0,50	1,14±0,55	1,99
27	Myosin (Mlc2)	muscle myosin	4	4	2	2	2	2	0,89±0,26	1,06±0,17	1,97
28	Actin 57B	muscle thin filament	4	3	2	2	2	2	1,00±0,42	0,92±0,46	1,87
29	Na channel 60E	plasma membrane	0	0	0	0	0	0	-0,16±0,25	1,49±1,99	1,82
30	CG4839	serine/threonine kinase	0	0	0	0	0	0	-0,48±0,95	1,60±1,74	1,82

Table II. The thirty muscle-related probesets with a higher increase (determined as the average change among the differenct microarrays in fold) in both ISS and RPM conditions is presented. Each probeset is identified by its gene title (note that some genes are represented by two probesets), GO cellular component at the left. The six columns in the middle indicates if the probeset expression is significally increased in the 0,1,2,3 or 4 of the 4 ISS comparisons, as well as for the four RPM comparison or just if it is present in 0,1 or 2 of each group microarrays. When a probeset is increased in the four comparisons (shaded) is considered to be increased in that condition. The left columns indicate the variation by Signal Log Ratio in ISS and RPM and its average fold change.

Some gene groups are altered in microgravity but half them show and increase and the other a decrease, as for example cytoskeleton components or peptidases (in this case serine-endopeptidases appears increased but ubiquitin or trypsin mediated proteolisys are decrased on microgravity). The main gene groups that shown a decrease expression in microgravity are related with the whole cell signaling process including cell surface receptors, G proteins, MAPKinases, transcription factors and chromatin remodeling components. Are also decreased the mechanisms of protein folding, phosphorilation/dephosporilation or glycosilation and also the apoptosis, cell death and different types of immune response genes including heat shock proteins.

4. DISCUSSION

This first analysis seems to indicate that several groups of genes are clearly affected by the microgravity environment and although some differences are found between the ISS and the RPM (simulated microgravity) they are not so severe as they looks like. Some genes groups have worth a special attention, because are not easy to relate with a direct microgravity response, for instance muscular, phototropic or ecdysone induced genes. They increase in microgravity together with a overall increase in mitochondrial components suggest a developmental delay in microgravity if we compare our results with previously published life cycle microarrays in *Drosophila* [5]. In Table II we present the particular data of the increased muscular genes in *Drosophila*, especially well-known in our laboratory, in order to test the accuracy of our results. We can observe that most of the gene increases have been detected, exception are Paramyosin gene (clearly increased but not in one of the samples) and TpnT (up held) successfully detected with one probeset but not with the other. Tm1 and some actins are increased in both situations but only detected for ISS.

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SUPPLEMENTARY MATERIAL. TABLE containing the 15 probesets with a higher variation in expression (Expressed as Signal Log Ratio average of the four comparisons ± standard desviation) in the six groups.

INCREASED IN ISS

Gene Symbol	Description	SLR ISS	SLR RPM
Eig71Ef	Ecdysone-induced	6,97±2,14	0,31±0,39
PGRP-SB2	peptidoglycan recognition	5,28±0,64	-1,91±1,41
Eig71Eg	Ecdysone-induced gene	4,74±0,43	-0,48±0,37
CG7924	Unknown	4,58±0,93	-1,53±1,21
Eig71Ed	Ecdysone-induced gene	4,32±0,17	-2,24±0,55
18S rRNA	Ribosomic RNA	3,93±2,44	0,01±0,77
CG7906	Unknown	3,82±1,12	-2,62±0,64
18S rRNA	Ribosomic RNA	3,74±2,3	-0,71±2,36
Eig71Ec	Ecdysone-induced	3,43±0,89	-1,53±1,04
CG15756	Unknown	2,99±0,58	0,37±0,4
CG4151	Unknown	2,32±0,54	-0,8±0,8
CG13069	Unknown	2,26±0,9	1,03±0,31
CG8329	Serine-peptidase	2,19±0,34	-0,93±0,85
CG30031 /// CG30025	Gamma Trypsin	2,14±0,64	-1,47±2,15
yellow-e	Unknown	2,04±1,27	1,14±0,59

DECREASED IN ISS

Gene Symbol	Description	SLR ISS	SLR RPM
jumu	Chromatin binding	-2,2±0,69	-1,36±0,15
CG12075	Transcription factor	-2,22±1,34	-1,9±0,73
inturned	Unknown	-2,3±1,05	-1,64±0,76
Osi12	Unkown	-2,33±0,97	-0,18±1,68
CG10077	Enzyme	-2,42±0,18	-0,62±0,72
CG33067	DNA binding	-2,46±0,77	-0,95±0,77
TepII	Unknown	-2,48±0,8	-1,04±0,41
CG32549	Unknown	-2,57±0,23	-2,31±1,16
LIMK1	Protein kinase	-2,61±0,53	-1,11±0,72
CG7294	Unknown	-2,66±0,55	1,29±0,8
kkv	Chitin synthase	-3,25±0,29	-1,09±0,41
CG13023	Transcription factor	-3,47±1,98	-3,55±3,52
CG8981	Unknown	-3,55±0,43	-0,87±0,79
RhoGEF4	cell cycle regulator	-3,59±0,48	-1,64±1
Ih	voltage-gated ion channel	-3,99±0,45	-1,55±1,1

INCREASED IN ISS & RPM

Gene Symbol	Description	SLR ISS	SLR RPM
CG5945	Unknown	3,49±1,94	4,87±1,06
CG13314	Unknown	2,82±0,75	3,09±0,73
CG14573	Unknown	2,76±1,67	3,59±1,59
CG5023	calponin-like	2,27±1,16	3,42±0,85
CG14022	enzyme	2,67±0,73	3,06±0,55
CG17298	Unknown	2,93±2,12	4,18±0,75
CG13239	Unknown	2,19±0,94	3,21±0,54
CG16749	endopeptidase	2,31±0,35	2,99±1,15
CG11912	endopeptidase	2,57±1,04	3,67±1,88
CG12388	endopeptidase	1,65±0,3	3,2±1,05
CG14568	Unknown	1,78±0,67	2,85±0,75
TpnC25D	Calcium binding	2,39±0,78	2,46±0,52
CG7443	Unknown	3,07±2,37	3,02±0,53
TpnC47D	Calcium binding	2,31±0,76	2,46±0,5
CG6974	endopeptidase	1,58±0,75	3,21±0,23

DECREASED IN ISS & RPM

Gene Symbol	Description	SLR ISS	SLR RPM
CG16772	Unknown	-1,66±0,58	-2,99±0,7
CG32499	Unknown	-2,37±0,61	-2,72±0,29
Eig71Ek	Ecdysone inducible	-1,66±0,92	-5,11±1,67
CG15678	Unknown	-1,64±0,87	-4,64±1,82
Hsp70B/ Hsp70A	chaperone	-2,08±0,74	-5,23±2,52
CG4726	Na^{2+}/PO_4^{3-} cotransporter	-2,6±0,66	-4,04±0,88
Hsp67Ba	heat shock protein	-2,28±0,84	-4,28±1,18
CG5326	Unknown	-2,65±0,55	-3,57±1,75
CG16886	Unknown	-2,3±0,75	-2,86±0,97
Mmp2	metalloendopeptidase	-1,98±0,23	-3,5±0,49
CG12505	Unknown	-3,12±0,27	-2,77±0,38
CG32499	Unknown	-2,64±0,7	-2,97±0,28
Ote	Otefin	-3,49±0,83	-3,4±0,57
CG13050	Unknown	-3,06±0,73	-3,82±1,18
CG33006	Unknown	-3,27±0,45	-5,03±1,89

INCREASED IN RPM

Gene Symbol	Description	SLR ISS	SLR RPM
Acp53C14a	Signal transduction	1,34±1,87	6,23±0,78
Acp53Ea	peptide hormone	0,76±2,01	5,89±0,71
Mst57Da	Male-specific RNA 57Da	2,59±2,43	4,12±0,63
Acp26Ab	peptide hormone	-0,35±1,36	4,04±1,58
Acp62F	Accessory gland peptide 62F	0,3±0,43	3,84±0,87
CG3239 /// CG32762	endopeptidase	2,52±1,44	3,66±0,35
CG8562	zinc Carboxypeptidase	1,67±1,21	3,62±1,35
CG9672	endopeptidase	2,69±0,26	3,53±0,68
CG18302	Unknown	0,56±2,02	3,46±0,93
CG33091	Unknown	1,01±1,07	3,41±1,19
CG14820	carboxypeptidase A-like	1,63±0,6	3,1±0,62
Cyp4d20	cytochrome P450	2,13±1,32	3,07±0,99
CG1791	structural protein	1,58±0,58	3,02±0,89
CG10911	Unknown	1,33±1,49	2,97±1,31
CG8012	Unknown	1,1±0,91	2,75±0,5

DECREASED IN RPM

Gene Symbol	Description	SLR ISS	SLR RPM
PpD5	Unknown	-0,65±0,39	-3,07±0,19
ImpE2	Ecdysone-inducible	0,36±0,72	-3,09±1,9
CG7802	Unknown	-0,57±0,4	-3,13±1,73
---	Unknown	-0,89±1,07	-3,24±1,67
CG7669	Unknown	-1,7±1,27	-3,3±1,63
Lcp4	Larval cuticle	-0,28±0,37	-3,32±0,86
Hsp26	Heat shock protein	-0,64±0,45	-3,4±2,06
CG3355	serine protease	1,65±0,74	-3,41±0,67
Osi6	RNA-directed DNA polymerase	1,06±0,23	-3,44±1,28
CG13215	Unknown	-0,53±0,83	-3,47±0,42
Tom	Twin of m4	-0,54±0,38	-3,48±0,72
Iswi	nucleosome remodelling	-1,17±0,55	-3,57±1,7
CecA2	antibacterial response	-0,21±1,37	-3,79±1,07
Cht1	Chitinase	-1,97±1,44	-3,89±0,81
CG18117	Chitinase	0,04±0,55	-3,9±1,93