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Biology

Unit 5: Respiration, Internal Environment, Coordination and Gene Technology

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Microgravity elicits reproducible alterations in cytoskeletal and metabolic gene and protein expression in space-flown *Caenorhabditis elegans*

Introduction

1. Spaceflight-induced muscle atrophy poses a significant risk for astronaut health and mission performance. Accordingly, it is a major obstacle for manned deep-space exploration. In contrast to observations on the Earth, exercise countermeasures employed during spaceflight have proven ineffective for maintaining skeletal muscle mass. Although the optimal exercise and nutritional strategies might simply have not yet been developed, there is growing evidence that muscle may be intrinsically sensitive to the effects of spaceflight *per se*. For example, vascular and cardiac muscles undergo atrophy, without reported muscle unloading, and cultured embryonic muscle synthesizes less protein as a direct consequence of spaceflight. However, the molecular events regulating these changes remain unclear.

2. Here we aimed to identify the mechanisms that are triggered in response to microgravity using the nematode *Caenorhabditis elegans*. In this space experiment (CERISE; *C. elegans* RNA Interference in Space Experiment), we synchronously cultured L1 larvae-stage animals to adulthood in liquid media for 4 days either in microgravity or 1-G (gravitational acceleration unit) centrifuge conditions onboard the Japanese Experiment Module of the International Space Station (ISS). The nematode larvae were launched to the ISS onboard the Space Shuttle Atlantis STS-129 on 16 November 2009, cultures were initiated on 20 November 2009, and the post-cultivation frozen samples were returned by the Space Shuttle Endeavour STS-130 on 21 February 2010.

3. *C. elegans* is an ideal model organism for studies of the physiological effects of space environments because of its small size, short life cycle, and because of its common use on Earth as a model organism for human medical pathologies. The first examinations of the effects of the space environment, including cosmic radiation, on *C. elegans* were performed using essentially standard laboratory culturing techniques with agar plates. *C. elegans* can mate, reproduce, and undergo embryonic development during spaceflight, and radiation-induced mutations can be monitored during spaceflight experiments. During the Dutch Soyuz mission DELTA to the ISS in April 2004, an international collaboration of laboratories carried out the "the first International *C. elegans* Experiment in Space" (ICE-First). In the ICE-First experiment, we found that muscle-, metabolic-, and aging-related genes showed 10–20% lower expression levels in response to spaceflight. These studies were carried out in a chemically defined medium, which allowed study of the effects of surface tension in flight but which significantly alters *C. elegans* life history in a manor akin to dietary restriction.

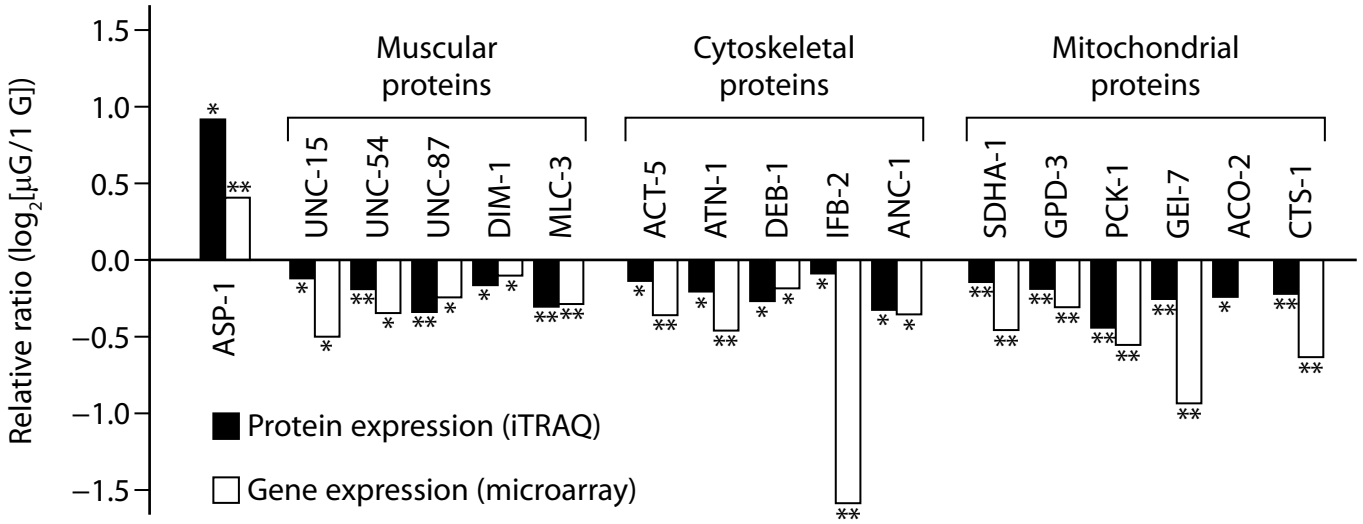
4. In this study, we wished to confirm that gene and protein expression changes observed in ICE-FIRST were not unique to the operational issues associated with that spaceflight, were not owing to the use of a non-standard laboratory diet, and were owing to exposure to microgravity. To achieve these goals we flew worms on a different launch system, cultured them in a different segment of the ISS, froze them on orbit, used the standard nutritionally rich bacterial diet, and cultured them both in microgravity and on a 1-G centrifuge onboard the ISS. In our previous study ICE-First, worms exposed to microgravity showed ~20% lower expression of thick filament components such as myosin heavy chain and paramyosin upon return to Earth. In the current study CERISE, changes in the expression of these same genes were found in the worms grown in microgravity versus in those grown at 1 G (changes were 53–71%). These results confirm that the past reported gene and protein expression changes for *C. elegans* during spaceflight are not unique to specific operational constrains or diet, and are owing to microgravity. In addition to alterations in cytoskeletal genes we have observed reproducible changes in metabolic genes including sirtuin, which is also known to influence ageing. Consistent with the gene and protein expression changes we also found lower fat accumulation and shorter body length in the microgravity-exposed worms. Last, alterations in the movement of the microgravity worms were observed. These results demonstrate a reproducible set of *C. elegans* gene and protein expression changes in response to microgravity and provide the basis for future mechanistic studies of how microgravity impacts muscle alteration and metabolism in *C. elegans* and possibly astronauts.

Results

Proteomic and transcriptomic analysis of space-flown *C. elegans*

5. To identify proteins that were differentially expressed in the nematodes during spaceflight, we quantitatively analyzed the expression levels of 475 proteins by matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry. This analysis revealed significantly increased expression of 16 proteins and significantly decreased expression of 43 proteins in microgravity ($P \leqslant 0.05$). Of the 16 proteins with increased expression, the expression of ASP-1 (aspartic protease) was increased 1.89-fold ($\log_2[\mu G/1\ G] = 0.92$) with the remaining proteins only slightly increased; these include proteins involved in protein synthesis such as ribosomal proteins and translation elongation factors (Figure 1). Gene expression analysis confirmed the downregulation of muscle-related proteins such as the myosin heavy chains, troponins, and intermediate filaments in the microgravity-cultured worms (Figure 1). Importantly, the pattern of downregulation of expression of muscle genes such as myosin heavy-chain genes *myo-3* ($\log_2[\mu G/1\ G] = -0.89$) and *unc-54* ($\log_2[\mu G/1\ G] = -0.49$) as well as the paramyosin gene *unc-15* ($\log_2[\mu G/1\ G] = -0.62$) were reproducibly observed in both the current experiment and the previous flight experiment.

Figure 1



Quantitative analysis of protein (iTRAQ) and gene expression (microarray) levels in space-flown worms. The levels of certain muscular, cytoskeletal, and mitochondrial proteins and an aspartic protease (ASP-1) were significantly altered in worms cultured under microgravity conditions. $*P \leqslant 0.05$, $**P \leqslant 0.01$, Student's *t*-test for analyses.

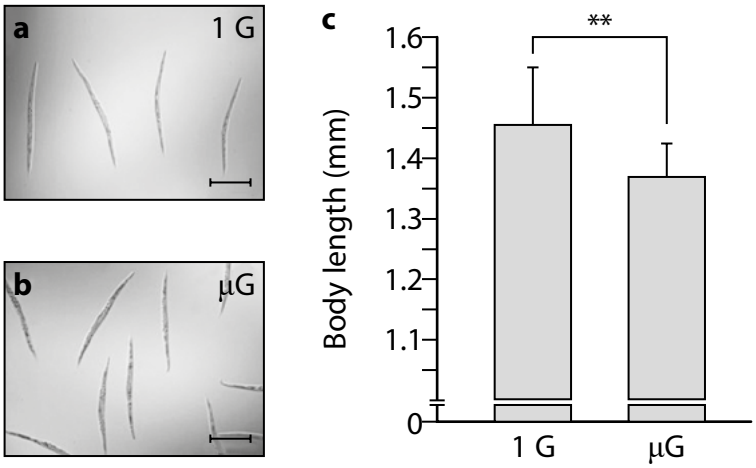
6. Other cytoskeletal components also displayed decreased expression during culture in microgravity, for example ACT-5 (actin), ATN-1 (α-actinin), DEB-1 (vinculin), IFB-2 (intermediate filament), and ANC-1 (nuclear and mitochondrial anchorage protein. In addition, significantly reduced levels of metabolic proteins were observed during culture in microgravity, for example, components involved in glycolysis (GPD-3), gluconeogenesis (PCK-1: phosphoenolpyruvate carboxykinase), and the glyoxylate cycle, a variation of the tricarboxylic acid (TCA) cycle (GEI-7). Moreover, the levels of components of the electron transport chain (SDHA-1) and the TCA cycle (ACO-2 and CTS-1) also significantly decreased during spaceflight. DNA microarray analysis confirmed these decreased protein expression levels in response to microgravity and suggested other energy metabolism gene expression level changes in microgravity. For example, genes encoding NADH dehydrogenase (complex I) and succinate dehydrogenase (complex II) displayed lower gene expression levels in the worms cultured in microgravity versus those cultured on the 1-G centrifuge (complex I: log_2[μG/1 G] = –0.18 to –1.03, complex II: log_2[μG/1 G] = –0.56 to –0.76). Similarly, ATP synthase expression was down (log_2[μG/1 G] = –0.38 to –0.76).

7. Whereas DNA microarray analysis suggests that major muscle components, cytoskeletal elements, metabolic genes, and mitochondrial electron transport genes largely are downregulated in microgravity, some genes in these broad classes appear to be upregulated. Notably, a sirtuin gene, *sir-2.1* encoding SIRT-1 protein, was upregulated. Sirtuin proteins deacetylate histones and several transcriptional factors, resulting in the activation or repression of target genes, and the sirtuin family may act as a global regulator of tissue health in response to diet and other stimuli. It was reported that *sir-2.1* is involved in longevity and anti-aging of *C. elegans*, and that *abu-6*, *abu-7*, and *pqn-5* are downstream targets that are negatively regulated by *sir-2.1*. Our microarray data indicate not only that the expression level of *sir-2.1* significantly increases in microgravity but also that the downstream target gene expression levels significantly decrease under microgravity condition; notably, significant changes in the same directions of expression alteration were also noted on our previous flight. Real-time quantitative PCR (qPCR) analysis confirmed the increased expression of *sir-2.1* and the decreased expression of downstream genes, which are negatively regulated by *sir-2.1*, including *abu-6*, *abu-7*, and *pqn-5*.

Body length and fat accumulation of nematodes cultured in microgravity

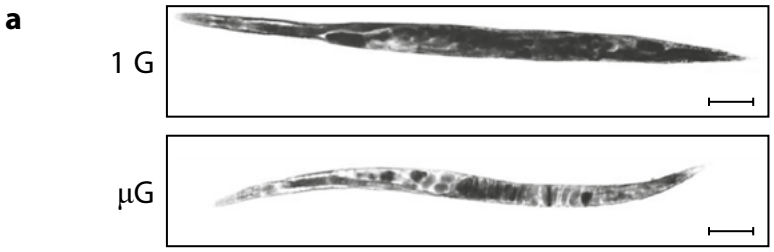
8. As proteomic and microarray analyses demonstrated decreased muscle and metabolic gene expression in microgravity, body lengths of a small subset of worms not used for omic analyses were microscopically measured. The body length of worms cultured in microgravity were slightly but significantly decreased by ~5.5% versus worms cultured on the centrifuge (μG: 1.37 ± 0.053 mm, 1 G: 1.45 ± 0.094 mm, $n = 15$ worms per group, $P \leqslant 0.05$; Figure 2). The data from the omics analyses suggest that the space-flown worms had lower energy metabolism capacity. Several molecules involved in fat metabolism are downstream targets of sirtuin signaling. Our DNA microarray analyses indicated that microgravity induced the decreased expression of *fat* genes encoding fatty acid desaturases and *lbp* genes that are involved in lipid binding activity (Figure 3c). The *sir-2.1* downstream target genes *fat-7* and *lbp-6* were among the downregulated transcripts ($\log_2$[μG/1 G] = –0.38 ($P = 0.01$) and –0.47 ($P = 0.00$), respectively). Therefore, we assessed lipid stores in a small subset of worms not used for omic analysis. Sudan Black staining indicated that the accumulation of fat in microgravity-cultured nematodes was significantly reduced compared with 1-G cultured worms (Figure 3a,b).

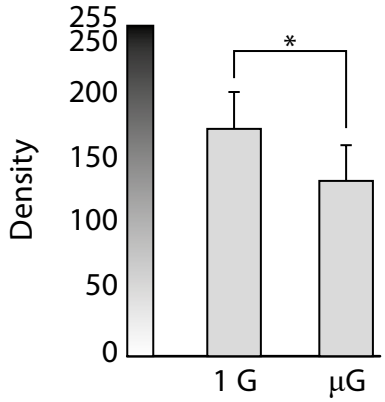
Figure 2



Alteration of body length of space-flown *C. elegans*. (a) Microscopic image of worms grown on the 1 G centrifuge for 4 days. (b) Microscopic image of worms grown in microgravity for 4 days. The worms in both conditions had normal levels of eggs in their bodies. (c) The mean of body length of the worms grown at 1 G was 1.45 ± 0.09 mm, and for microgravity was 1.37 ± 0.05 mm, respectively ($n = 15$, means ± s.d.). $^{**}P \leqslant 0.01$, Welch's *t*-test. Scale bars indicate 0.5 mm.

Figure 3





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gene name	$\log_2$[μG/1 G]	*p*-value
fat-1	−0.82	0.00
fat-3	−0.72	0.00
fat-4	−0.12	0.02
fat-5	0.57	0.00
fat-6	0.19	0.01
fat-7	−0.38	0.01
lbp-1	0.49	0.01
lbp-2	0.39	0.01
lbp-3	−0.49	0.01
lbp-5	−1.23	0.00
lbp-6	−0.47	0.00
lbp-7	−1.47	0.00
lbp-8	0.75	0.01
lbp-5	0.21	0.01

Altered fat storage in microgravity-cultured *C. elegans*. (a) Images of Sudan Black staining of accumulated fat in *C. elegans*. Worms grown onboard the 1 G centrifuge (upper image) and in microgravity (lower image) are displayed. Scale bars indicate 100 μm. There was less fat accumulation in microgravity-cultured worms compared with 1 G controls. (b) Density measurements of Sudan Black stained worms (as described in Methods). The mean densities were 135.36 ± 28.12 in microgravity-cultured worms and 176.55 ± 27.61 in 1-G cultured worms (means ± s.d.). $*P \leqslant 0.05$, Student's *t*-test. (c) Alteration of fat-related gene expression determined by microarray analysis. *P*-values as indicated in the panel, Student's *t*-test.

Effect of microgravity on swimming behavior of *C. elegans*

9. To examine the swimming behavior changes of *C. elegans* during spaceflight, we analyzed the swimming motion of worms grown under microgravity or 1 G on the ISS for 4 days. As shown in Table 1, wavelength showed no difference between microgravity and 1-G cultured worms. However, frequency of swimming was significantly different, and this likely caused a significant difference in wave velocity. Amplitude was measured at the point of 0.1 L, and no significant difference was observed. These results suggest that microgravity induced lower beating frequency and slower wave velocity without changing the shape feature of movement, i.e., the movement of the worm became slower under microgravity.

Table 1: Characteristics of moving behaviors of space-flown nematodes

Culture condition	μG (*n* = 6)	1 G (*n* = 3)	*P*-value
Wavelength (L)	2.29 ± 0.71	2.07 ± 0.30	>0.1
Frequency (Hz)	1.53 ± 0.64	2.61 ± 0.12	<0.01
Wave velocity (L/s)	3.19 ± 0.76	5.39 ± 0.64	<0.01
Amplitude (rad/L)	4.09 ± 0.76	4.75 ± 0.40	>0.1

Discussion

10. It is well known that microgravity exerts considerable effects on physiological processes. Muscle atrophy is one of the main concerns for astronauts during spaceflight. To counter atrophy, astronauts are forced to perform several exercises such as using an ergometer. In 2004, we had the opportunity to conduct an experiment using the nematode *C. elegans* on the ISS. Nematodes flown in microgravity for ten days exhibited reduced gene expression of muscle-related molecules. Both gene and protein expression levels of myosin heavy chain and paramyosin, main components of thick filaments in invertebrates, significantly decreased in space-flown worms. In the present study, we also observed lower expression levels of both the major components for myofilament assembly such as myosin heavy chain and paramyosin. This repeated observation suggests that these changes are caused by spaceflight and not operational, technical, or dietary differences between these two spaceflight experiments.

References and Acknowledgements

http://www.nature.com/articles/npjmgrav201522

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