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Review of the results from the International *C. elegans* first experiment (ICE-FIRST)

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Abstract

In an effort to speed the rate of discovery in space biology and medicine NASA introduced the now defunct model specimen program. Four nations applied this approach with *C. elegans* in the ICE-FIRST experiment. Here we review the standardized culturing as well as the investigation of muscle adaptation, space biology radiation, and gene expression in response to spaceflight. Muscle studies demonstrated that decreased expression of myogenic transcription factors underlie the decreased expression of myosin seen in flight, a response that would appear to be evolutionarily conserved. Radiation studies demonstrated that radiation damaged cells should be able to be removed via apoptosis in flight, and that *C. elegans* can be employed as a biological accumulating dosimeter. Lastly, ICE-FIRST gave us our first glimpse at the genomic response to spaceflight, suggesting that altered Insulin and/or TGF-beta signaling in-flight may underlie many of the biological changes seen in response to spaceflight. The fact that the results obtained with *C. elegans* appear to have strong similarities in human beings suggests that not only will *C. elegans* prove an invaluable model for understanding the fundamental biological changes seen during spaceflight but that it may also be invaluable for understanding those changes associated with human health concerns in space.

1. Introduction

In an attempt to increase the rate of progress in space biology, NASA decided to adopt a model organism approach, an approach that as of this writing has been shelved in favor of experimentation largely upon humans alone. The premise of this model organism approach was that if standardized culturing conditions were employed then multiple investigators, working on multiple questions, could be accommodated within a single flight. ICE-FIRST was initiated by Centre National d'Études Spatiales (CNES) as an attempt to demonstrate the feasibility and utility of such an approach with the nematode *Caenorhabditis elegans. C. elegans* was one of a number of model organisms selected for NASA's "model specimen" approach. A brief overview of the rationale for the use of eukaryotic genetic model organisms in general and *C. elegans* in specific for space biology and medicine was provided in the description of ICE-FIRST (Szewczyk et al., 2008). Notably, since that publication an additional Nobel prize in Chemistry (development of the green fluorescent protein) and a Lasker Award (discovery of microRNA) were awarded for work first done in *C. elegans. C. elegans*

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researchers have a community ethic and a *C. elegans* database (AceDB, www.acedb.org) (Eeckman and Durbin, 1995) has spawned a number of resources that are maintained by the community (Antoshechkin and Sternberg, 2007). Key websites include a PubMed indexed text (www.wormbook.org) and an information repository that uses AceDB as a backend master database (www.wormbase.org). Reviews for the non-specialist can also be found with Corsi's 2006 review (Corsi, 2006) being a good starting point.

C. elegans had previously been employed to study the impacts of spaceflight upon biology, typically by one investigator attempting to address a specific question. These studies involved four spaceflights. The study on STS-42 showed that male animals mated successfully and the full life-cycle of *C. elegans* occurred in space without obvious abnormal developmental process (Nelson et al., 1994b). A study, which started on STS-42 and finished on STS-76, reported an increased rate of mutation as the direct effect of cosmic radiation (Hartman et al., 2001, Nelson et al., 1994b). Sadly, an experiment carried out by American high school students on STS-95 yielded no results and both the ground control and flight animals died, probably due to anoxia. On the final flight (STS-107) before ICE-FIRST NASA began the process of establishing a new inflight culture system for *C. elegans* (Szewczyk et al., 2005). ICE-FIRST continued this process and also demonstrated the potential utility of a model specimen approach in space biology (Szewczyk et al., 2008).

ICE-FIRST was a collaborative effort among four nations: France, the United States, Japan, and Canada. During ICE-FIRST, *C. elegans* were grown in or on culturing media as described below. Fifty-three sets of animals were prepared and loaded in flight hardware in Toulouse, France 5–7 days prior to launch, as described by Szewczyk and colleagues (2008), before they were flown on the European Space Agency's 2004 Delta Mission which lasted approximately 10 days. Parallel sets of transport and ground control animals were also utilized (Szewczyk et al., 2008). The standardized culturing as well as the investigation of muscle adaptation, space biology radiation, and pattern of gene expression in response to spaceflight serve as the conceptual framework of this review.

2. Validation of *C. elegans* Maintenance Media (CeMM) for spaceflight

Traditionally, *C. elegans* is almost exclusively grown on Nematode Growth Medium with the uracil auxotrophic bacterium *Escherichia coli* OP50 as a food source (NGM) (Brenner, 1974). This standardization of culturing has proved essential in understanding the biology of *C. elegans* on Earth (www.wormbook.org). In parallel to these efforts a minority of researchers pursued developing a chemically defined medium for *C. elegans* growth (CeMM: *C. elegans* Maintenance Medium); after many years of effort this medium was completed and published (Lu and Goestsch, 1993). This alternative culturing medium was selected by NASA as ideal for growing *C. elegans* in space for a number of reasons. First, unlike traditional culturing, which requires astronauts to frequently transfer worms to new plates, CeMM allows worms to be cultivated without transfer for at least four times as long. Second, the new medium, in a liquid form, can be used for automated culturing and experimentation, thereby removing the need for astronaut intervention (e.g. the medium would also be useful for unmanned satellite and/or interplanetary missions). Third, the chemically defined nature of the medium removes the theoretical health concerns to astronauts surrounding the use of a bacterial food for *C. elegans* in flight. Fourth, the chemically defined nature of the medium removes the concerns of altered *E. coli* metabolism in flight being a confounding variable in determining the effects of spaceflight on *C. elegans*. Lastly, the new medium, in a liquid form, removes the potential masking of microgravity effects due to the surface tension forces (10,000–100,000G) that hold worms to plates.

Based on these reasons and in anticipation of NASA's requirement that future *C. elegans* experiments in space to employ CeMM, ICE-First used CeMM instead of NGM. The investigators showed that the growth and development of space flown *C. elegans*, using either liquid or solid CeMM, was essentially the same as *C. elegans* grown under similar conditions in the laboratory. These results were as anticipated based upon previous demonstrations of normal growth and development in flight for animals grown on NGM (Nelson et al., 1994a) and from the extrapolation of normal growth and development in flight for animals grown on both NGM and solid CeMM (Szewczyk et al., 2005); the later data were generated by extrapolation due to the delayed recovery of samples following the tragic breakup of the Space Shuttle Columbia. The demonstration of grossly normal growth and development suggests that CeMM presents opportunities for further studies including the possibility of automated experiments in flight. This is absolutely critical for experiments on deep space flights to other planetary bodies.

C. elegans and humans have a number of basic similarities, both on the anatomical and genomic level. They both have muscles, a nervous system, integument, gut, and a reproductive system. Both have approximately 20,000 genes and approximately 60% of *C. elegans*' genes have human homologues. In addition, both have completely known genomic DNA sequence, which allows for indepth bioinformatic analyzes. Thus, it is anticipated that *C. elegans* can be used to understand more detailed, non-population lethal, effects of spaceflight on humans. Indeed, this has been previously demonstrated by showing that *C. elegans* display an altered rate of mutation in response to spaceflight (Hartman et al., 2001), and by some of the results detailed in the sections below.

3. Effect of spaceflight associated radiation

Radiation is perhaps the most significant medical challenge associated with manned spaceflight (Hagen, 1989, Stewart and Lujan, 1993). For this reason, *C. elegans* has previously been used as a genetic model with which to understand the mutagenic consequences of spaceflight. On a pair of flights to low Earth orbit (LEO), Nelson and colleagues showed a small but increased rate of mutation during spaceflight that was directly attributable to high energy radiation particle bombardment (Nelson et al., 1994b). LEO orbits are protected by the Earth's magnetosphere and therefore are not subject to the full effects of solar and cosmic radiation that flights to other planetary bodies will encounter. This is good for astronauts on the International Space Station but further deep space studies are needed to analyze radiation effects of flights beyond Earth orbit. In an attempt to expand our understanding of the biological effects of radiation during spaceflight in LEO, the investigators undertook studies designed to further develop *C. elegans* as a model system for studying the biological effects of spaceflight associated radiation. These investigations were focused on two themes: apoptosis and the mutational effect(s) of spaceflight.

3.1 Occurrence of apoptosis in spaceflown C. elegans

Apoptosis plays an important role in normal metazoan development and among its normal functions is the elimination of cells that have suffered DNA damage that could lead to cancer or, in the case of germ cells, heritable genetic disorders. This would be extremely important on spaceflights because of increased exposure to cosmic and solar radiation with its concomitant increase in DNA damage. Therefore, the investigators examined these two apoptotic processes: physiological and checkpoint apoptosis. These studies were carried out using wild-type and *ced-1* mutant animals, as described by Higashitani and colleagues (2005).

Physiological and checkpoint apoptosis were examined by observing if the process occurred normally in cells in the regions of the germ line associated with each process. The analysis

uncovered no differences between the spaceflight and control animals. Wild-type animals had normal levels of apoptosis and the *ced-1* animals had the expected increase in cell corpses. Together these results demonstrate that the rate of apoptosis and engulfment of cell corpses occur normally during spaceflight. The investigators also attempted to confirm this by using Affymetrix microarray analysis of checkpoint and apoptosis related genes. The microarray analysis of checkpoint and apoptosis related genes showed similar gene expression for both spaceflight and ground control animals thereby again failing to demonstrate a difference in apopotosis in response to spaceflight.

In summary, these data demonstrate that physiological apoptosis and checkpoint apoptosis occurred normally in spaceflown *C. elegans*. This suggests that animals, human beings included, retain the ability to eliminate cells that failed to repair DNA lesions caused by cosmic radiation during the spaceflight and may explain why cataracts and not cancer are a leading long term side effect of spaceflight in American astronauts (Longnecker et al., 2004).

3.2 Mutational effect of spaceflight in C. elegans

Four different approaches were employed to measure mutational changes that occurred during the Canadian Space Agency sponsored part of the spaceflight, as described by Zhao and his colleagues (2006). These are: 1) Capture of mutations in the *unc-22* gene; 2) Alterations in telomere length; 3) Poly-G/poly-C tract integrity; 4) Capture of mutations in the *eT1* balancer system, which balances 1/6 of the entire genome (Rosenbluth and Baillie, 1981). The investigators failed to detect any statistically significant alterations in spaceflown worms. With regard to actual detection efficacy, a slight increase in telomere length was noted in the spaceflown versus ground control animals, no mutations were detected in *unc-22* in either set of animals, no deletions were detected in G-tracts in either set of animals, and 13 and 11 lethal strains were recovered from the spaceflown and ground control *eT1* animals, respectively. The lethal strains recovered fell into three complementation groups for the spaceflown animals and eight for the ground controls. These results suggest that the mutagenic effects of radiation in LEO are small.

Taken together, these data show that the *eT1* balancer system is the best of the four systems tested for detecting mutagenic events resulting from radiation events in flight. Additionally, the *eT1* system allows one to capture, maintain and recover mutations in spaceflown *C. elegans*, thereby allowing one to investigate in a laboratory on Earth mutagenic events captured during flight. Lastly, these data appear to imply that one could use the *C. elegans eT1* system as a “biological dosimeter” that could be employed to monitor the long term accumulation of mutagenic events in flight (Zhao et al., 2006).

4. Muscle adaptation in spaceflight

The molecular basis underlying muscle adaptation during spaceflight is yet to be fully understood. Previous data in tissue culture studies have shown decreased muscle gene transcription in response to spaceflight (Vandenburgh et al., 1999), and these changes are sufficient to account for the muscle atrophy observed in whole mammals. Therefore the ICE-FIRST investigators undertook a study of the response of *C. elegans* muscle to spaceflight to determine if this *in vivo* model can be used to gain insight into the molecular mechanisms underlying spaceflight-induced muscle atrophy and corroborate the past *in vitro* work.

4.1 Alteration of muscle development in C. elegans in response to spaceflight

The effect of spaceflight upon *C. elegans* muscle development was examined. The investigators assayed gene expression, post flight movement, and muscle histology.

Gene expression was methodically analyzed using DNA microarrays, Real-time quantitative PCR, 2D gel electrophoresis and Western blots. The myosin heavy chain genes (A, B, C, D), body-wall myogenic transcription factor HLH-1(CeMyoD) and pharyngeal myogenic transcription factors (PEB-1, CEH-22 and PHA-4) showed decreased expression as assayed by microarray, PCR, and Western blot. The muscle specific genes tropomyosin (*lev-11*), troponin C (*tnc-2*), and troponin T (*tnt-2, -3, -4*) also showed significant decreases in expression as assayed by microarray, whereas no significant changes, as assayed by microarray, were noted in actin (*act-1, act-2, act-3, act-4*). The investigators suggested that the microgravity associated with spaceflight resulted in decreased expression of MyoD, which in turn resulted in decreased expression of myosin heavy chain. Of note, this model appears to be true for both *C. elegans* body-wall muscle, which allows voluntary movement, and pharyngeal muscle, which rhythmically contracts like cardiac muscle. The investigators also reported that similar changes are known to occur in spaceflown vertebrates, suggesting an evolutionarily conserved mechanism underlies altered development of muscle during spaceflight.

Post-flight movement was examined for defects using recorded video footage taken within two hours of landing. The results showed that 10 randomly selected spaceflown animals were significantly different from 10 randomly selected ground control animals, indicating defects in movement. While the previously described changes in gene expression can account for post-flight movement defects, a direct link between these genes and the post-flight movement defect was not shown by the investigators. Additionally, it should be noted that a second group of randomly selected spaceflown animals, from a different population did not display significant post-flight movement defects. A comparison of gene expression changes revealed depression of “neuromuscular” genes correlated with the postflight movement defect (Selch et al., 2008) as animals with a postflight movement defect had depressed expression of neuromuscular genes while animals without a postflight movement defect had no depression in neuromuscular genes. While the results of multiple independent analyses are consistent with the notion that depressed expression of muscle genes can result in a post-flight movement defect, the direct casual link and reason for individual differences in depression of muscle genes remains to be established.

Muscle histology of spaceflown wild-type *C. elegans* was also carried out. Phalloidin and anti-paramyosin staining of post-flight animals revealed no gross morphologic changes, including no change in the width of the fibers. While these results indicate a lack of spaceflight induced “atrophy” of the fibers, the animals used for histologic analysis did not show the same depression in paramyosin and myosin heavy chain B (Adachi et al., 2008) as the animals that had a post-flight movement defect (Higashibata et al., 2006), thus reinforcing the idea that there are individual specific differences that underlie spaceflight-induced alterations in muscle. If this is true than *C. elegans* would appear to mirror the situation in human beings where there appears to be great variability in muscle atrophy in response to spaceflight (Fitts et al., 2001, Adams et al., 2003).

In summary, ICE-FIRST demonstrated that *C. elegans* has altered muscle development in response to spaceflight with changes that appear similar or identical to those observed in mammals. Specifically, decreased MHC expression, presumably due to decreased MyoD expression was noted and these changes correlated with a post-flight movement defect. Intriguingly, as is the case for astronauts, there were population differences noted in the muscle adaptation to spaceflight. Taken together these results suggest that *C. elegans* can be used to study an evolutionarily conserved mechanism that underlies spaceflight-induced muscle alterations.

4.2 Alteration of muscle development in a paramyosin mutant

In addition to investigating the effect of spaceflight upon *C. elegans* muscle development in wild-type animals, the effect was also assessed in *unc-15 (e73)*, paramyosin, mutant animals.

Paramyosin interacts with MHC A, an isoform of myosin heavy chain in striated muscle. The investigators assayed muscle histology and various muscle protein levels.

As with wild-type animals, histologic study of *unc-15 (e73)* animals was conducted using phalloidin and anti-paramyosin staining. In both ground control and spaceflown *unc-15* animals, deformed thin filaments and the aggregated paracrystalline form of paramyosin were noted. However, in spaceflown worms partially formed normal paramyosin filaments were also observed. Additionally, the spaceflown animals displayed a normal muscle filament to body-width ratio that was not observed in the ground control animals. Thus, spaceflight appears to have partially rescued the histologic defects of the paramyosin mutant.

Again as with wild-type animals, Western blots were used to assess the levels of paramyosin, myosin heavy chains B and C, actin, and tropomyosin III. Spaceflown *unc-15* mutant animals displayed increased levels of paramyosin and myosin heavy chains relative to both ground controls and spaceflown wild-type animals. In contrast, actin remained the same and tropomyosin III was slightly depressed, although the depression was not statistically significant. Thus, as with wild-type animals, the thick and thin filament proteins showed different effects in response to spaceflight. However, unlike wild-type animals, which showed decreased thick filament proteins in response to spaceflight, *unc-15* animals showed increased thick filament proteins. These observations suggest two things. First, spaceflight has a differential effect on thick and thin filaments regardless of mutations in a thick filament gene. Second, spaceflight allows animals to better compensate for a mutation in the thick filaments by increasing thick filament gene expression.

Together the histologic and Western blot data from *unc-15* animals suggest that altered muscle development, induced by spaceflight, allows partial rescue of the defects induced by the mutation. A direct elucidition of the functional consequences and the mechanism underlying the rescue remains to be demonstrated. If spaceflight does indeed rescue the functional consequences of mutations in muscle proteins this suggests that muscle damaged in flight may be better able to repair than muscle damaged on Earth, a view that runs counter to the current conventional wisdom. However, while we have presented the *unc-15* data as spaceflight having “rescued” the effects of the mutation the investigators have correctly pointed out that there may be concerns with this apparent rescue. Specifically, their data can also be interpreted to show that increased muscle protein degradation, a required component of muscle atrophy, is found in the mutants vs. wild-type. If the investigators are correct, this reinforces the currently widely held view that muscle damaged during spaceflight may not be properly able to repair. Future studies are clearly needed.

5. Analysis of the genomic response to spaceflight

Not only was *C. elegans* the first animal to have its genome sequenced, it has the most completely annotated sequence. This combined with the fact that the human genome has also been sequenced makes *C. elegans* a premier model for genomic studies. Therefore, the ICE-FIRST investigators embarked upon one of the first analyses of the genomic response to spaceflight. Genomics is a young field and thus methodology is a major driver both in interpretation of results and in fundamental versus applied discovery. For these reasons the investigators employed a variety of techniques and attempted to look at both fundamental and applied questions. As discussed in the preceding sections, the investigators employed a targeted approach to analyzing alterations in gene expression as they related to other findings such as altered muscle development, apoptosis and normal developmental process of *C. elegans.* The investigators also employed a number of unbiased analyses in an effort to understand the global changes *C. elegans* gene expression induced by spacflight. Lastly, because another group of investigators conducted a similar experiment for another genomic model, *Drosophila*

melanogaster, on an ESA mission prior to ICE-FIRST, a comparative genomic analysis was done.

5.1 Gene transcription response to spaceflight in C. elegans

The gene transcription response to a 10-day spaceflight was examined using two full genome microarray platforms, the Stanford *C. elegans* cDNA array (Kim et al., 2001) and the Affymetrix array (Hill et al., 2000). Three independent spaceflight and ground control population samples were compared to standard culture populations on Earth. The investigators used a log2 change of >2 as a cut-off mark ($p<0.05$ for 1.3–1.7 fold) across as many replicates as possible (as many as three and as few as one). In an effort to understand the biological function/meaning of the observed changes in gene expression the authors plotted their results against the genomic self-organizing map (Kim et al., 2001) using $p<0.001$ to assign significance to changes within “mountains” on the map.

The first population analyzed (author’s sample 4) had significant increases in expression for only 28 genes and decreases in expression for 24 genes relative to expression under standard culturing conditions. When the results were adjusted to account for changes in the sister ground control population and looked at in terms of “biological function”, this population appears to have decreased expression of amino acid and lipid metabolism genes, decreased expression of collagen genes, and increased expression of fatty acid oxidation genes. In the second population (author’s sample 22), there was a significant increase in expression for only 25 genes and a decrease in expression for 28 genes relative to standard culturing conditions. When the results were adjusted to account for changes in the sister ground control population and looked at in terms of “biological function”, this population has decreased expression of neuromuscular genes, decreased expression of amino acid metabolism genes, decreased expression of collagen genes, and increased expression of heat shock stress response genes. The third population (author’s sample 23), had a significant increase in expression for only 16 genes and a decrease in expression for 23 genes compared to standard culturing conditions. Adjusting to account for changes in the sister ground control population and looking at “biological function”, this population has decreased expression of neuromuscular genes, decreased expression of intestinal genes, decreased expression of amino acid metabolism genes, decreased expression of collagen genes, and increased expression of muscle and heat shock stress response genes. The investigators noted that the analysis of genes identified as showing reproducible changes were confirmed in a fourth population of animals using RT-PCR, Western blotting and Affymetrix microarray (Higashibata et al., 2006).

Three themes emerge from the microarray data. First, there are few genes that show reproducible changes in response to spaceflight when assayed in multiple replicates of a population. This may mean that gene responses to spaceflight are small and/or that the number of genes altered in response to spaceflight is relatively small. This is not particularly surprising because gross development, apoptosis (Higashitani et al., 2005), and DNA repair (Zhao et al., 2006) all appeared normal in response to spaceflight. Second, there are some genes that show reproducible changes in response to spaceflight both within replicates of a population and across populations. Notably, the genes that have these highly reproducible changes are largely metabolic (decreased) and stress response genes (increased). Additionally, the bulk of these genes are thought to be regulated by two signaling pathways that the worm uses to sense and respond to the external environment, Insulin and TGF-beta. Third, within the class of genes that gave reproducible changes in expression it is possible to identify genes that change in only two of three populations during spaceflight; for example, decreased expression of neuromuscular genes. This may suggest differential sensitivity of populations to spaceflight or, as the authors state, population differences in exposure to multiple vs. individual stressors associated with spaceflight. This may also explain why one population of spaceflown worms

had a post-flight movement defect while one did not and also why there is wide variability in muscle loss in astronauts and cosmonauts (Fitts et al., 2001, Adams et al., 2003).

Together the results paint a semi-convincing picture of how *C. elegans* modulates gene expression in response to spaceflight. First, worms use the same signaling pathways as on Earth to sense and modulate gene expression in response to spaceflight, namely Insulin and TGF-beta. Second, the highly reproducible changes in gene expression are metabolic genes. Third, there are individual differences in response to spaceflight that may be explained in terms of individual difference in metabolic demand due to multiple stressors in flight (for example spaceflight vs. spaceflight and hypoxia). It remains to be seen if these underlying messages will also emerge from spaceflown mammals.

5.2 Common changes in global gene expression in Drosophila melanogaster and C. elegans

The comparative analysis is described by Leandro and his colleagues (2007). The authors used three different approaches to analyze the similarities and differences in the gene expression and found only a few with common gene expression changes. Given the strict statistical criteria applied and generally small reproducible set of genes shown to change in spaceflown *C. elegans*, as discussed above, this result is not particularly surprising. However, six genes were found to have a common response to spaceflight. They were all identified as having decreased expression in both organisms:

- **i.** T04F8.2 (*C. elegans*) or CG9304 (*D. melanogaster*): yet to be characterized but possibly a G-protein coupled receptor
- **ii.** F10E7.4, *spon-1* (*C. elegans*) or CG6953, fat-spondin (*D. melanogaster*): a fat-spondin-like protein involved in cell adhesion and notably important for neuron and muscle development (Woo et al., 2008)
- **iii.** C01B7.4, tag-117 (*C. elegans*) or CG31243, cpo or CG32717, sdt or CG1864, Hr38 (*D. melanogaster*): yet to be characterized but possibly a membrane associated guanylate kinase involved in scaffolding and signaling in postsynaptic cells
- **iv.** F35H12.4 (*C. elegans*) or CG7004, fwd (*D. melanogaster*): yet to be characterized but possibly a phosphatidylinositol kinase
- **v.** F31F6.6, *nac-1* (*C. elegans*) or CG3979, Indy (*D. melanogaster*): a sodium coupled dicarboxylate transporter
- **vi.** F13D12.2, *ldh-1* (*C. elegans*) or CG10160, ImpL3) (*D. melanogaster*): a lactate dehydrogenase.

Clearly the finding of only six genes that show reproducible changes in response to spaceflight in two different organisms is an under-representation of the conserved genomic responses and is expected for both methodological reasons (discussed by the authors (Leandro et al., 2007) and for statistical reasons (e.g. the highly stringent selection criteria employed by the authors)). However, the fact that all of the identified genes are either metabolic and/or neuromuscular “signaling” genes suggest that there is indeed a conserved metabolic alteration in these two species (and likely in mammals) that involves both a metabolic shift and altered neuromuscular development/function. It remains to be seen if there are master genes (e.g. transcription factors) controlling the response to spaceflight (for example, as scaffolding proteins SPON-1 and/or TAG-117 could hypothetically be “gravity sensors” upstream of an unidentified transcription factor). Regardless, it seems reasonable to speculate that altered metabolic gene expression in response to spaceflight underlies the bulk of the changes observed in response to spaceflight and it is clear that future work is needed to demonstrate the causal links, if any, between these six genes and phenotypes associated with spaceflight.

6. Conclusions

We have reviewed the results from ICE-FIRST and have attempted to illustrate that *C. elegans* is a robust system for studies addressing the impact of spaceflight upon biology. A variety of investigations carried out during ICE-FIRST, by researchers from four nations, have added to our knowledge of the biological response to spaceflight. With respect to radiation effects during spaceflight, ICE-FIRST demonstrated that radiation damaged cells should be able to be removed via apoptosis in flight and that *C. elegans* can be employed as a biological accumulating dosimeter. With respect to spaceflight effects on muscle, ICE-FIRST demonstrated that decreased expression of myogenic transcription factors underlie the decreased expression of myosin seen in flight, a response that would appear to be evolutionarily conserved. Lastly, ICE-FIRST gave us our first glimpse at the genomic response to spaceflight, suggesting that altered Insulin and/or TGF-beta signaling in-flight may underlie many of the biological changes seen in response to spaceflight. The fact that the results obtained with *C. elegans* appear to have strong similarities in human beings suggests that not only will *C. elegans* prove an invaluable model for understanding the fundamental biological changes seen during spaceflight but that it may also be invaluable for understanding those changes associated with human health concerns in space.

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