The next phase of life-sciences spaceflight research
Harnessing the power of functional genomics

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Recently we demonstrated that the effectiveness of RNA interference (RNAi) for inhibiting gene expression is maintained during spaceflight in the worm Caenorhabditis elegans and argued for the biomedical importance of this finding. We also successfully utilized green fluorescent protein (GFP)-tagged proteins to monitor changes in GFP localization during flight. Here we discuss potential applications of RNAi and GFP in spaceflight studies and the ramifications of these experiments for the future of space life-sciences research.

Spaceflight appears to induce metabolic changes in all species yet studied, many of which are suggested to have detrimental consequences for crew health and performance.1 These (mal)adaptations represent a major obstacle preventing the world’s space agencies from safely achieving their common goal of sending humans on long duration deep-space exploratory missions, and ultimately the human habitation of other planetary bodies. To overcome this obstacle we must first understand the molecular mechanisms regulating spaceflight-induced alterations and, second, utilize this knowledge to effectively target efforts to develop therapeutic strategies. To date detailed mechanistic investigations and molecular therapeutic interventions have largely been lacking. This is primarily due to the numerous technical and logistical difficulties associated with conducting spaceflight research, which has limited most studies to being observational in nature.

Utilizing model organisms to further our understanding of cellular processes is a crucial element of biological research, both on Earth and in space. The soil nematode Caenorhabditis elegans (C. elegans) is one such model system which is especially suited to spaceflight experiments as they can be cultured automatically,2 are inexpensive, require minimal storage room, have a wealth of genetic and molecular tools available (www.wormbook.org) and, importantly, have been shown to recapitulate various alterations reported for humans and rodents.3 While descriptive experiments have established C. elegans as an in vivo system in which to help understand the biological effects of spaceflight,3-8 in order to begin to harness C. elegans as a translational experimental system we must now perform hypothesis-driven mechanistic and interventional experiments.

Facilitating this aim, in a recently published study,9 we report that the effectiveness of RNA interference (RNAi) at silencing target genes is maintained during spaceflight. Additionally, we showed that RNAi can be used to alter the subcellular localization of green fluorescent protein (GFP)-tagged proteins. We believe this study provides the necessary proof in principle that mechanistic and interventional experiments are feasible during spaceflight. Specifically, we have demonstrated that two, Nobel prize-winning techniques can be utilized during spaceflight: RNAi for studying the functional effects of gene knock-down10 and...
fluorescent protein-tagged molecules for studying in vivo sub-cellular changes in specific proteins. The very simple experimental design employed to demonstrate the efficacy of RNAi and GFP technologies in space illustrates the relative ease with which these important tools can be incorporated into future experiments to study the molecular mechanisms underpinning the biological alterations induced by spaceflight. Importantly, these studies could be conducted in real-time during spaceflight by acutely treating animals with RNAi and examining the effects on GFP-tagged proteins via direct imaging. For example, we have previously reported that in addition to changes in muscle gene expression, changes in genes known to be controlled by insulin signal- ing are observed in response to spaceflight. Additionally, as the intermediary signaling components, the insulin-controlled transcriptional regulator FOXO (DAF-16 in *C. elegans*) has been grown onboard the International Space Station using an automated culturing system that included light microscopy for growth and observation of an animal in low Earth orbit. J R Soc Interface 2011; In press.

While this provides a single example of how RNAi and GFP technologies may be employed on future spaceflight experiments, such an approach may also be used on a much wider scale. Using microarray analyses, previous spaceflight studies in *C. elegans* have reported lists of genes that appear to be affected by short-term and long-term (unpublished) spaceflight. Using this information, the functional consequences of RNAi against genes that show altered expression in flight could be assessed to identify key regulatory genes. Such experiments would be relatively cheap, logistically simple and have important ramifications for delineating the molecular mechanisms that control organismal adaptations to spaceflight. Furthermore, while our results have been limited to *C. elegans*, there is no reason to pre-suppose that RNAi could not be used effectively in both cultured cells and higher organisms in space. Thus, the sorts of experiments that we suggest for *C. elegans* are likely feasible in higher organisms as well. Clearly the experimental strategies we propose should speed the process of scientific discovery and translational science/ medicine in space.

Recently, *C. elegans* have been grown for six months onboard the International Space Station using an automated culturing system that included light microscopy capabilities. Adapting this system to allow for fluorescent microscopy would be relatively straightforward. Therefore, experiments similar and/or identical to what we propose above could be incorporated on unmanned, deep-space missions in order to understand and prevent deleterious biological consequences of these flights. Ultimately, one could also consider remotely cultivating and experimenting upon a multi-cellular animal on another planet.

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