Yang Zhao, Martin Jones, David Baillie and Ann Rose

Developing an integrating biological dosimeter for spaceflight

Exposure to harmful radiation is one of the major threats to human beings in outer-space; however, the biological consequences of long term exposure are not well understood. It would be useful to have a means of measuring the effect of space radiation on a living organism during space flights. We conducted a pilot project as part of the International Caenorhabditis elegans Experiment First Flight (ICE-First) project on the International Space Station (ISS). Using a mutational capture system, the eT1 balancer, along with other mutation detection systems, we analyzed the mutational effects of the 11 day mission. Upon recovery, classical genetic approaches and comparative genomic hybridization (CGH) microarrays were used to isolate and characterize mutant strains. Although in this short period of time, as expected no increase in mutational background was observed, we were able to demonstrate the potential of this system for longer-term measurement of biological damage. A six-month exposure experiment using the same system is currently in progress on the ISS. The relative simplicity and robustness of this model system demonstrate its potential for use as a biological dosimeter.

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Authors

Yang Zhao, Ann Rose
Department of Medical Genetics, University of British Columbia
Life Sciences Centre, Room 1364-2350 Health Sciences Mall
Vancouver, BC, Canada V6T 1Z3

Martin Jones, David Baillie
Department of Molecular Biology and Biochemistry
Simon Fraser University
8888 University Drive, Burnaby, BC Canada V5A 1S6.

1. Introduction

A major safety concern of human activities in outer-space is the exposure to harmful radiation in the environment. It is well known that radiation can induce mutational damage which can result in diseases such as cancer, and death due to radiation poisoning. Much of our understanding of the effect of radiation on living animals comes from genetic analysis of model organisms. As a well-established model organism, *C. elegans* is an excellent system for the studies of mutational effects of radiation since it has a DNA damage response similar to humans (Reviewed by O'Neil and Rose [1]). *C. elegans* has been used extensively to study the phenotypic consequences of exposure to radiation. Rosenbluth and colleagues originally established the *eT1* balancer system and used it to genetically analyze the mutational effects in *C. elegans* by X-irradiation and γ-irradiation [2,3]. Using this system, along with other detection methods such as chromosome non-disjunction and *unc-22* mutagenesis, Nelson and colleagues carried out further investigations on the mutational effects of *C. elegans* under different types of radiation such as high and low linear energy transfer (LET) ionizing radiation [4,5]. Features of *C. elegans*, such as small size, simplicity of maintenance, and variety of research resources, have made it a model system for space biology studies (reviewed by Johnson and Nelson [6]; Zhao et al. [7]). *C. elegans* has been previously sent into space and it has been shown that it can reproduce and develop normally during the spaceflight [8]. Analysis on dormant worms exposed to natural space radiation during the same flight revealed an elevated mutation rate compared to ground controls [9]. More recent studies by Hartman and colleagues compared mutations in a single *C. elegans* gene induced by accelerated iron particles and low earth orbit space radiation, indicating that high LET charged particles are an important mutagenic component of space radiation [10]. While previous studies have focused on the immediate effects of natural space radiation (single generation), we were more interested in understanding the long term effects (multiple gen-

erations) of space radiation inside of the space vessels. Ultimately it would be desirable to have an easily maintained living system as a dosimeter that can be used to measure the biological effects of exposure over long periods of time. In our project, we have taken advantage of the defined liquid medium, the *C. elegans* Maintenance Medium (CeMM), which can be used to maintain growth of worms for several months without intervention [11].

The International *Caenorhabditis elegans* Experiment First Flight (ICE-First) on board of Delta Mission (19 to 30 April 2004) was a project using *C. elegans* as a model organism to

study the biological effects of a short duration spaceflight (11 days in the International Space Station ISS). The research projects included muscle proteins, genomics, ageing, development, apoptosis, gravity sensing, and radiation biology [Szewczyk et al., manuscript submitted]. Our contribution to the ICE-First project was to investigate the potential of *C. elegans* as an integrating biological dosimeter to examine the mutational effects of increased radiation exposure in the space environment [12].
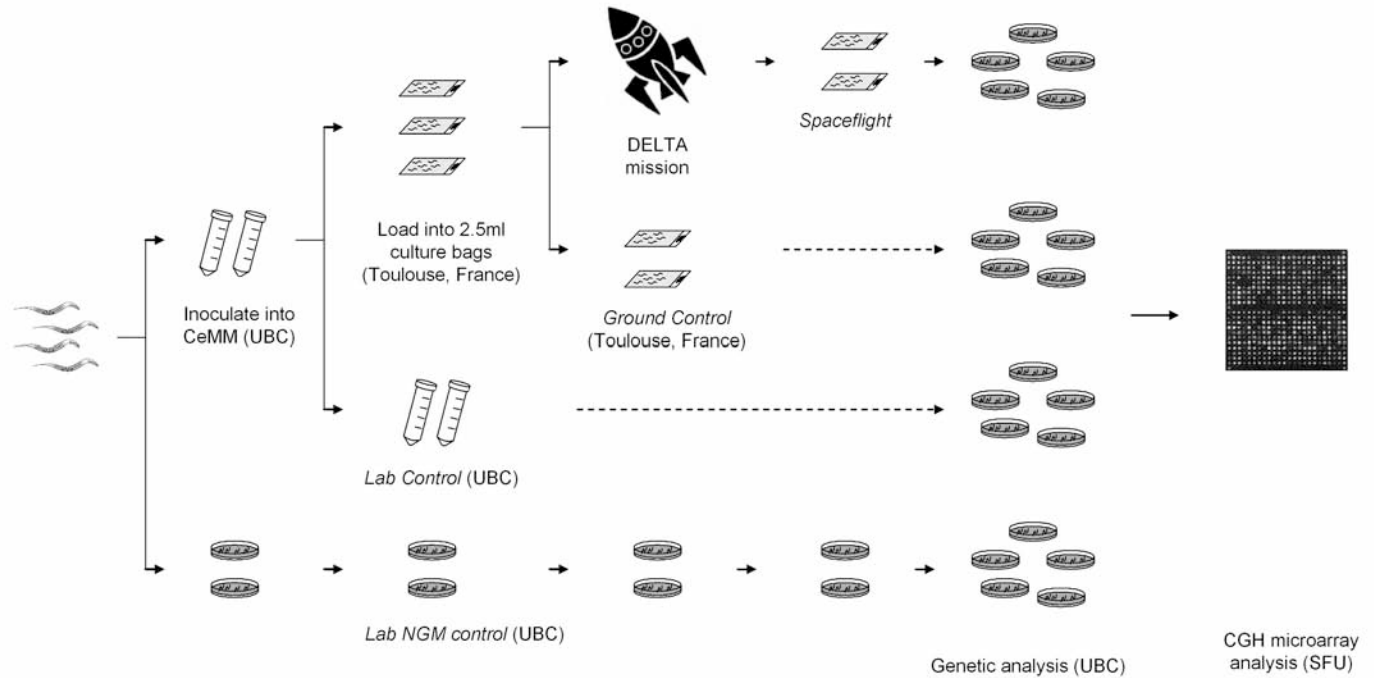

Fig 1. Flow chart of the experimental procedure. Worms were divided into four samples: Spaceflight, Ground Control, Lab Control, and Lab NGM Control (shown in italic)

Fig 2. a) Culture bags that contain C. elegans in CeMM; b) Temperature controlled incubator

2 Experiments and Results

2.1 Experimental procedure

2.1.1 Adaptation of worms to the CeMM

CeMM is a defined liquid medium in which *C. elegans* can be maintained without intervention[11], and was used for the ICE-1 mission. Worms are required to be sterilized to get rid of contaminations like yeast and bacteria before being inoculated into the liquid media. To remove contaminants the animals were treated with alkaline hypochlorite[13]: Worms on NGM plates were allowed to grow until young gravid adults formed the majority of the population. Adults were washed off the NGM plates by water and transferred into a centrifuge tube. The animals were then washed 2-3 times with water and collected by centrifugation. Alkaline hypochlorite solution was then added into the tube and allowed to stand for 10-12 minutes. Adult worms and bacteria were killed and dissolved while eggs were retained alive. The bleaching solution was diluted out of the culture with several washes in sterile water and the pellet of eggs collected by centrifugation. Eggs were re-suspended in sterile water and then inoculated into CeMM.
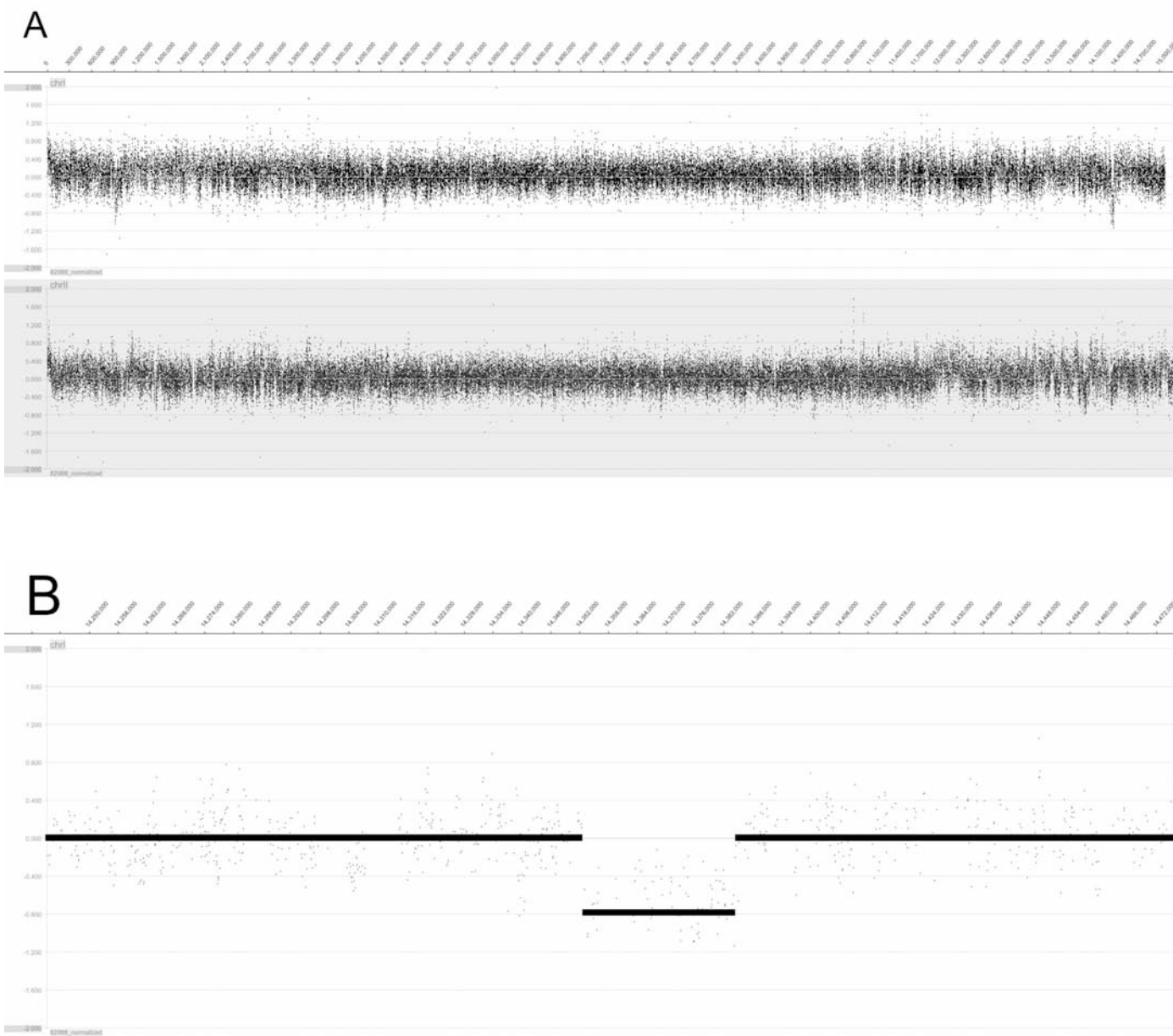

Fig 3: Example of a fine-tiling Array CGH of a C.elegans deficiency strain shown in the SignalMap browser software. A) Data set of LGI and LGII b) Detection of a ~31 Kb deletion in LGI

2.1.2 Spaceflight and controls

The experimental and control animals were processed as described in Fig. 1. Worms in CeMM were grown in 25ml Falcon centrifuge tubes in the lab at UBC before they were split into pools. Some pools were sent to France while the remaining animals formed the UBC Laboratory Control pool. In France, worms were packaged into 2.5ml culture bags (Fig. 2a). 6 bags were sent to the launch site in Baikonur, Kazakhstan and flown to the International Space Station while the remaining 6 bags were retained in Toulouse, France as the Ground Control (Szewczyk, personal communication). Spaceflight worms were maintained in a temperature controlled incubator during the flight (Fig. 2b). During the experiment, worms were maintained at 20˚C except the spaceflight samples were at 12˚C for 5 days at Baikonur before the launch.

2.1.3 Laboratory NGM control

A population of worms were setup on NGM plates as described by Rosenbluth [2,3] as the Laboratory NGM control. The number of generations was calibrated using the generation time: 7-10 days in CeMM[11] and 4 days on NGM at 20˚C, in order to reproduce on NGM the number of generations spent in CeMM for the experimental samples. These animals were grown on 15cm agar Petri plates seeded with OP50 E. coli. and were transferred to a new plate every second week by agar chunk method to minimize founder effects.

2.2 Mutational analysis

Four mutation detecting systems were tested in this analysis: telomere length, poly-G/poly-C tracts integrity, mutation events in unc-22 gene, and the eT1 balancer system. None of these four system suggested significant difference in the mutation rate between spaceflight and control samples during the short time on the ISS. The first three systems did not demonstrate enough sensitivity for detecting mutational events during the spaceflight. The eT1 balancer system successfully detected and captured 17 mutations, from both experimental and controls samples, demonstrating its usefulness for measuring mutational damage during spaceflight [12]. Genetic characterization of mutations isolated by the eT1 balancer system was performed and both single gene mutations and chromosomal rearrangements were shown to be recovered by this system[12].

2.3 Microarray analysis of mutations isolated by the eT1 balancer system

DNA samples of mutations isolated by the eT1 balancer system were prepared for CGH (Comparative Genomic Hybridization) Microarray analysis. Genomic DNA was isolated from mixed stage worms grown on 4 large NGM plates. Worms were first digested with protinase K followed by an RNase treatment and phenol/chloroform extraction. DNA samples were tested for purity and diluted to a concentration of 250ng/ml before being delivered to Nimblegen Systems Inc. for the CGH microarray analysis.

For our initial experiments a C. elegans whole genome tiled CGH array was used. This chip, produced by Nimblegen Systems Inc., is based on the Wormbase CE2, WS120 build and consists of 385,000 45-85mer probes spaced at a median separation of 137bp across the entire genome. Sample QC, fluorescent dye labelling, array hybridisation and Data analysis are performed by Nimblegen Systems Inc. Test strains are labelled with Cy3 and hybridised to the array using Cy5 labelled N2 DNA as a reference. The array data are supplied in both raw and processed formats. The formatted data can be viewed and manipulated with Signalmap browser software (Fig-3).

3 Discussion and future directions

Physical detectors, although being able to record certain radiation types, cannot determine the biological effects of exposure. A biological assay is therefore needed for this purpose. In our experiment, a population of C. elegans was incubated in both the transit spacecraft (Soyuz Spacecraft) and the International Space Station (ISS) so that we could examine the mutational effects upon these animals within the human-living environment of spaceflight. While the other three systems we tested did not capture mutation events in this study, our analysis demonstrated the capabilities of eT1 balancer system in detecting, capturing, maintaining, and recovering the mutation events occurred during the spaceflight[12]. Extensive studies using this system have shown its sensitivity to mutagenesis, and dose-response curves of several types of radiation have been obtained [2-5,9,10]. This system has also been used to capture mutation events in C. elegans exposed to natural space radiation [9]. While this previous study focused on the effects of natural space radiation on dormant animals, we have demonstrated that the eT1 balancer system can also work for long-term multiple-generation studies using actively growing nematodes. The system is robust and easily maintained, providing the possibility of use in longer term missions. A six-month exposure experiment using our system is currently in progress on the ISS.

The ability of the eT1 balancer system to capture and maintain mutational events has made detailed post-flight analysis possible. Both single gene mutations and chromosomal rearrangements were captured in the experiment[12]. Classical genetic analysis of mutations isolated by eT1 balancer system is a fairly time-consuming process. Technological advances in genome analysis can now provide faster and more precise measurements of mutational effects. The eT1-captured mutants are currently being analyzed using CGH microarray analysis to examine the extent of the accrued damage. CGH analysis provides a reliable and cost effective method for rapidly assessing DNA alterations on a genome-wide scale. Samples recovered from space flight can be cultured, have their genomic DNA isolated, processed and analyzed within a few weeks post flight. This is a dramatic improvement over the months of mapping