WORMS IN SPACE? A MODEL BIOLOGICAL DOSIMETER

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INTRODUCTION

Although it is well known that radiation causes mutational damage, little is known about the biological effects of long-term exposure to radiation in space. Exposure to radiation can result in serious heritable defects in experimental animals, and in humans, susceptibility to cancer, radiation-sickness, and death at high dosages. It is possible to do ground controlled studies of different types of radiation on experimental animals and to physically measure radiation on the space station or on space probes. However, the actual biological affects of long-term exposure to the full range of space radiation have not been studied, and little information is available about the biological consequences of solar flares. Biological systems are not simply passive recording instruments. They respond differently under different conditions, and thus it is important to be able to collect data from a living animal. There are technical difficulties that restrict the placement of an experimental organism in a space environment for long periods of time, in a manner that allows for the recovery of genetic data. Use of the self-fertilizing hermaphroditic nematode, *Caenorhabditis elegans* offers potential for the design of a biological dosimeter. In this paper, we describe the advantages of this model system and review the literature of *C. elegans* in space.

THE *C. ELEGANS* SYSTEM

C. elegans is a well-established animal model, which is easy to culture in a laboratory. Normally it is maintained on agar culture plates and fed a non-pathogenic bacteria. *C. elegans* is a self-fertilizing, effectively isogenic hermaphrodite which produces approximately 300 progeny from a single individual. Hermaphrodites have two X chromosomes while males, which are XO, are produced spontaneously as a result of X-chromosome loss or nondisjunction. Once mated by a male, a hermaphrodite produces out-cross progeny. This is a very useful feature for doing genetic crosses. The hermaphroditic life style is especially useful for maintaining animals over several generations, on for example, sustained missions. The complete cell lineage is known, including those cells genetically programmed to die (apoptosis). The worms are transparent at all developmental stages, making it possible to examine cell division and development in real time. *C. elegans* is a metazoan with a number of tissue types, such as nervous system, muscle, intestine and gonad. Many of the developmental and biochemical pathways are conserved with human. There is a large community of *C. elegans* researchers taking genetics-based approaches to understanding fully the biology of this organism. An introduction to the *C. elegans* system and literature has been reviewed in Riddle et al.(1997) and is available at a web site maintained by Leon Avery at http://elegans.swmed.edu/. At this site there is also information about up-coming meetings, abstracts, in-house publications, researchers' contact information, and current methodologies.

EXPERIMENTAL RESOURCES

The genome has been completely sequenced and consists of approximately 20,000 predicted protein-encoding genes (Sequencing Consortium 1998) and at least 50% of *C. elegans'* genes have human homologs (McKay, et al. 2004). A powerful approach for studying loss of gene function technology has recently been developed. Double-stranded RNA can be introduced be either injection into the worm or be feeding. The most widely used approach, developed by A. Fire's laboratory, involves feeding worms bacteria, which are producing double-stranded RNA for the gene of interest. The ingestion of the dsRNA causes inhibition of gene expression (RNAi) (reviewed in Fire et al. 1999). Libraries containing bacterial strains for most of the predicted protein-encoding genes have been constructed, and the phenotype for each of the genes observed (Fraser et al. 2000; Kamath, et al. 2003). In addition, the targeted deletion of specific genes has been undertaken by the*C. elegans* Reverse Genetics Consortium (a collaboration of the R. Barstead laboratory in the USA, the D. Moerman laboratory in Canada and the Y. Mitani laboratory in Japan), and requests and information are available at http://www.celeganskoconsortium.omrf.org/. An alternate approach to obtaining gene deletions is the use of transposable element insertion and excision by L. Segalat's laboratory in France. Mutant strains of the gene knockouts are made available by the Caenorhabditis Genetics Centre (CGC) (http://www.cbs.umn.edu/CGC/). The CGC maintains and provides thousands of strains generated by these and classical genetic approaches used by the hundreds of researchers that make up the *C. elegans* research community. In addition there are numerous rearrangements, duplications and deletion strains generated by the Genetic Toolkit Project, the cosmid transgenetic rescue project, and other researcher projects. A further aid to mapping mutants is the database descriptions of single nucleotide polymorphisms (SNP) between strains. Many of these SNP markers can be assayed by polymerase chain reactions (PCR) followed by

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restriction enzyme digestion, making this a fast efficient way to find the molecular basis of a desired phenotype (Wicks, et al. 2001; Swan et al. 2002). A large scale project to examine the expression profile of genes with human homologs (the "C. elegans II" project funded by GenomeBC, D. Baillie, p. comm.) has resulted in the construction of more than 2000 Promoter::GFP strains (McKay, et al. 2004). Large scale identification of protein interactions, the 'interactome' (Walhout et al. 2002; Reboul et al. 2003 Li et al., 2004), and stage-specific serial analysis of gene expression (SAGE) (Jones et al. 2001; McKay et al. 2004) have been developed. Access to these data and more can be obtained from WormBase http://www.wormbase.org/).

ANALYSIS OF GENE EXPRESSION

There are a number of resources for studying global gene expression in *C. elegans*. Microarray analysis was first used by Reinke et al. (2000) to produce a profile of germline expression. It has been used subsequently for a number of descriptions of differential gene expression, including a profile of the genes differentially expressed in the chemically defined CeMM media compared to the commonly used nematode growth media (NGM) (C. Conley & N. Szewczyk, NASA, p. comm.). In addition to microarray analysis, SAGE profiles of each of the developmental stages of *C. elegans*, including the dauer larvae stage have been done ((Jones et al. 2001; www.wormbase.org). Expression of individual genes which are turned on in specific developmental stages has been characterized by GFP promoter analysis (McKay, et al. 2004). In this case, a fluorescent reporter lights up when the gene is transcriptionally active (expressed). For example, a muscle-specific promoter expresses only in muscle (Fig. 1). The potential exists to adapt this approach to a reporter detection system in space, which would record which genes are turned on under particular conditions, for example, responses to lift-off and solar flares.

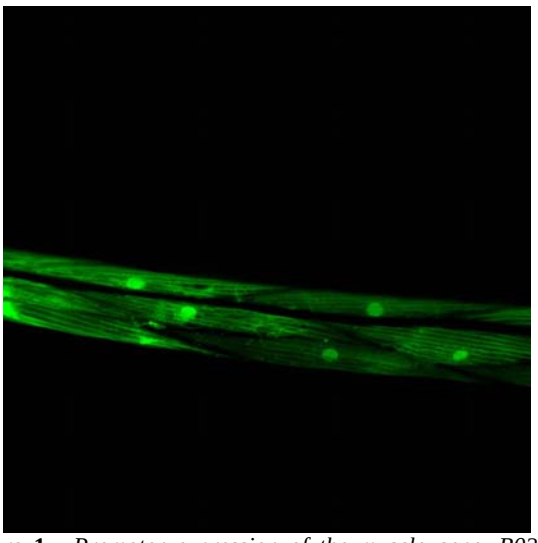

Figure 1. *Promoter expression of the muscle gene, B0228.4 using green fluorescent protein (GFP) as the reporter signal.*

REPAIR SYSTEMS

In the case of assaying the response to radiation, reporter analysis of repair genes would be of interest. *C. elegans* has a large number of repair genes which function in highly conserved pathways, that is the protein sequences are conserved with both yeast and higher organisms, including man. A review of the repair pathways (Rose, unpublished), and many other aspects of *C. elegans* biology, will be accessible in the upcoming worm book, C. elegans III which will be available on-line in 2005 at www.wormbase.org. Genes involved in responding to radiation damage were first identified by Hartman and Herman (1982). The review presents a summary of the genes for which mutant phenotypes have been described, including components of the pathways for nucleotide excision repair, mismatch repair, DNA damage checkpoint, non-homologous end joining, homologous recombination repair, and chromosomal structure surveillance. A broad perspective on genes involved in DNA repair has been gained using high throughput, genome-wide analysis of RNAi phenotypes (Piano et al., 2002; Kamath et al. 2003; Pothof et al., 2003; Lettre et al., 2004; vanHaaften et al.,) and protein interactions (B) and protein interactions (Boulton et al. 2002; Li et al.). As part of the interactome analysis, known proteins implicated in replication, nucleotide excision repair, mismatch repair, base excision repair, nonhomologous end joining, homologous recombination and checkpoint pathways were used in yeast 2-hybrid experiments to identify physical interactors in the predicted proteome (Boulton et al. 2002; Li et al. 2004). Components of the checkpoint signaling networks assemble into more complicated networks. Sensors, transducers and mediators are shared when generating different responses including chromatin remodeling, altered gene expression and DNA replication. The data demonstrate that many of the pathways are interrelated, and that pathway components exhibit previously unrecognized links between repair mechanisms and checkpoints.

A CHEMICALLY-DEFINED MEDIA

C. elegans also has many characteristics that make it an excellent model system for use in space. *C. elegans* reproduces as a self-fertilizing hermaphrodite, is small (adults are approximately 1 mm long), and thus easily grown in a small space. The life-cycle is short, approximately one-week under conditions at the International Space Station (ISS), and the progeny numbers high, a few hundred per hermaphrodite. The normal food source for *C. elegans* is bacteria; however for space experimentation it can be fed a chemically defined, axenic media (CeMM) adapted for space travel by C. Conley's laboratory at NASA, Ames (Lu and Goetsch (1993; Szewczyk N J et al. 2003). The worms will survive for several months in CeMM media, and can be transferred to fresh media and maintained apparently indefinitely. In addition, under conditions of overcrowding and limited food larvae can enter a dormant "dauer larva" stage. These dauers can survive for several

months at ambient temperature and will resume their normal life-cycle when introduced to fresh axenic media.

For experimental purposes, samples can be prepared either on the ISS or on the ground for subsequent data analysis. At the re-entry site the samples can be preserved alive in a frozen state in liquid nitrogen and be recovered later for biological analysis.

Figure 2. *Logo of the ICE-First mission.*

C. ELEGANS IN SPACE

Johnson and Nelson (1991) first proposed using *C. elegans* as a model system for space biology studies. Since then *C. elegans* has flown on several missions to Earth orbit, and was shown to develop and reproduce normally, making it an excellent model system for biological research in space. Nelson et al. (1989; 1994a; 1994b) investigated mutations induced by cosmic rays in *C. elegans* on Spacelab in low Earth orbit. Their analysis was for short-term (8 days) radiation exposure. Currently nothing is known about longer term exposure to the different types of radiation in space, nor about the effects of exposure to the range of radiation in the space environment. In his review, Nelson (2003) states that "The unique feature of the space radiation environment is the dominance of high-energy charged particles (HZE or high LET radiation) emitted by the Sun and galactic sources, or trapped in the Van Allen radiation belts. These charged particles present a significant hazard to space flight crews, and accelerator-based experiments are underway to quantify the health risks due to unavoidable radiation exposure". Recently, Nelson et al. (2002) examined the effect of different types of radiation, gamma rays, accelerated protons, and iron ions at the same physical dose. Using RT-PCR differential display and whole genome microarray hybridization experiments, they described unique transcriptional profiles for the different radiation treatments. The genes affected by each radiation species were associated with unique regulatory clusters, highlighting our limited knowledge of the biological responses to radiation exposure.

THE ICE-FIRST PROJECT

In this context, we took part in a recent International collaboration to use *C. elegans* as a model system for biological studies in space, ICE-First (Fig. 2). The project was coordinated by Michel Viso of the Centre Nationale d'Etudes Spatiales (CNES) with the help of the European Space Agency (ESA) and the Space Research Organization of the Netherlands (SRON) and was flown on the Dutch Science Mission (DSM) to the International Space Station during April 19-30 2004. Researchers from France, Japan, USA and Canada participated. The scientific projects included validation of liquid culturing in the space flight environment; studies on muscle protein growth and maintenance; whole genome microarraay responses to spaceflight; and morphology of larval development during space flight (Table 1). The effects on nematodes being grown for three to four generations in CeMM media and of being in space for the 10 days of the mission is being analyzed by A. Rose's laboratory for eT1-balanced mutations (Zhao et al., 'Spaced-out Worms' abstract available at http://elegans.swmed.edu/) and by D. Baillie's laboratory for changes in RNA expression (unpublished).

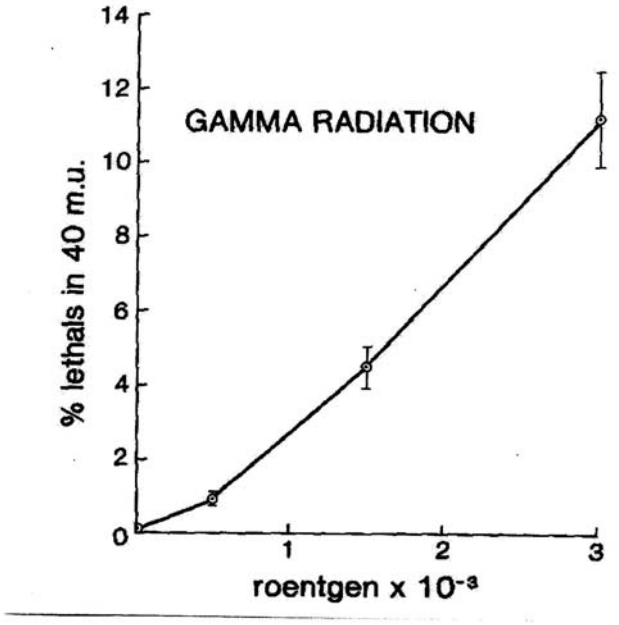

Figure 3. *Dose curve of gamma radiation. Data from Rosenbluth et al., 1983.*

ACCUMULATED MUTATION RATE MEASUREMENT

The most common type of easily identified mutation is that affecting expression of an essential gene (lethals). Lethals will not accumulate if normal animals are used. We have developed a system to measure the accumulated mutation rate, the eT1-system (Rosenbluth and Baillie, 1981; Rosenbluth et al., 1983) was used. *eT1(III;V)* (*eT1*) is a reciprocal translocation that recombinationally balances the left half of *Linkage Group V* [*LGV*(left)] and the right half of *Linkage Group III* [*LGIII*(right)] (Rosenbluth and Baillie 1981) which is nearly 20% of the

nematode's genome. *eT1(III)* breaks in *unc-36* thus giving *eT1* a visible phenotype. *LGV*(left) contains approximately 7% (23 m.u.) of the recombinational distance in the genome and approximately 10% of its DNA. It is relatively straightforward to calculate forward mutation rates using this system, and that has been done for mutagens routinely used in the laboratory, such as EMS and gamma radiation (Rosenbluth et al., 1983; 1985); formaldehyde (Johnsen and Baillie 1988) and UV radiation (Stewart et al., 1991). In the analysis of exposure to gamma radiation Rosenbluth et al., (1983) observed that at low doses, the curve was non-linear (Fig. 3). The data show that low doses of radiation are non-damaging, apparently due to repair mechanisms, which may be very good news for those spending long periods of time in space, if the radiation exposure is low level.

The *eT1* methodology can also be used to determine what types of mutations were generated. That is, are they putative point mutations or are they predominately small or large rearrangements? The mapping can determine if the new mutations occurred randomly or whether there were some mutational "hotspots". Any putative point mutations can also be identified as new alleles of known genes or as newly identified genes. The mutational rate and the types of mutations generated and preserved in a proposed accumulating dosimeter system could be quickly analyzed. There is a caveat to this that must be taken into account when analyzing mutations in the accumulating dosimeter. That caveat is the loss of worms that die as a result of mutations in essential genes due to purifying genetic selection. Therefore it is important to carefully screen for semi-viable and morphological mutations because these should not be eliminated as quickly through purifying selection. The majority of point mutations and rearrangements will be identified by analyzing semi-viable and morphological mutants. Many rearrangements will not include essential genes and thus not be subject to rapid purifying selection. Mutations induced by ionizing radiation are mainly rearrangements [Nelson et al. (1989; 1994); Rogalski, Moerman and D.L. Baillie (1982); Rosenbluth, Cuddeford and Baillie (1983)]. Therefore we expect that the majority of mutations captured in the accumulating dosimeter will be rearrangements, which could be efficiently analyzed for the entire genome using the method of comparative genomic hybridization similar to that developed by Ishkanian et *al*. (2004) for identifying minute genomic rearrangements in the human genome. The method consists of the construction of a DNA microarray containing overlapping BACs, PACs, YACs or in our case cosmid clones that cover the entire regions of interest. These microarrays are sensitive enough to detect single copy change and so can detect heterozygous deficiencies and duplications.

SUMMARY AND CONCLUSIONS

The wide variety of research resources, available for biological analysis in *C. elegans*, provide a promising backdrop for the development of specific systems to study the effects of traveling and living in space. A high priority could be the development of an accumulating dosimeter. NASA, for example, has called for protocols whose objective is to "determine the effects that long-term exposure to the space environment has over multiple generations in space".

In summary, the resources and knowledge of the *C. elegans* system make it an excellent biological model for both studies of gravitational effects on muscle gene expression and mutational consequences of radiation exposure.

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