RADIATION EFFECTS IN NEMATODES: RESULTS FROM IML-1 EXPERIMENTS

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ABSTRACT

The nematode *Caenorhabditis elegans was* exposed to natural space radiation using the ESA Biorack facility aboard Spacelab on International Microgravity Laboratory 1, STS-42. For the major experimental objective dormant animals were suspended in buffer or on agar or immobilized next to CR-39 plastic nuclear track detectors to correlate fluence of HZE particles with genetic events. This configuration was used to isolate mutations in a set of 350 essential genes as well as in the uric-22 structural gene. From flight samples 13 mutants in the uric-22 gene were isolated along with 53 lethal mutations from autosomal regions balanced by a translocation *eTl (III;V)*. Preliminary analysis suggests that mutants from worms correlated with specific cosmic ray tracks may have a higher proportion of rearrangement.s than those isolated from tube cultures on a randomly sampled basis. Flight sample mutation rate was approximately 8-fold higher than ground controls which exhibited laboratory spontaneous frequencies.

1NTRODUCI'1ON

The International Microgravity Laboratory # 1 (IML- 1) Spacelab mission using the ESA Biorack facility provided a capability for observing animal development as a function of gravity and for exposing sensitive germ cells to the natural radiation environment of space. The nematode *Caenorhabditis elegans* was used for both purposes as part of an experiment entitled "Genetic and Molecular Dosimetry of HZE Radiation"; it is also known as "US-1" or "Radiat" in Biorack and IML-1 mission documentation. The principal objective of the experiment was to measure the genetic damage induced by natural cosmic rays in animal cells in terms of rates and structures of induced lesions. Assessment of the fidelity of development and chromosome behavior under microgravity were secondary objectives and arc described in paper /1/.

HARDWARE DESCRIPTION AND METHODS

Hardware

The ESA Biorack is a miniaturized biological laboratory which takes the form of a Spacelab rack and provides a variety of services which include: incubators, 1 XG centrifuges, coolers, glove box and microscope /2/. Ancillary equipment provides passive temperature control to and from orbit in the shuttle mid-deck (PTCU's). All experiments utilizing Biorack arc housed in onc of two t ypcs of standardized containers called Type I or Type II containers. Eleven Type I and two Type 1 I containers were used for the *Radiat* experiment. Each is an anodized aluminum box with spring latches and alignment flanges to interface with Biorack centrifuges and storage racks. The Type I containers were placed in Biorack incubator A (at 22°C and at gravity levels of O or 1), in the Biorack cooler at 4° C, or in a Nomex® nylon belt with pouches attached to the Spacelab tunnel via Velcro® patches (a low shielding area selected to maximize radiation exposure). For the latter samples temperature control was provided by the overall Spacclab/Shuttle life support system and varied cyclically between 20 and 26° C as reported by an automatic temperature recorder which accompanied the samples.

Type I containers contained dormant nematode larvae in lexan tube suspension cultures or growing populations on thin films of agar seeded with *E. coli* bacteria controlled with antibiotics (see /1/). Accompanying the tube cultures were LiF thermoluminescent detector chips and CR-39 plastic nuclear track detectors /3/. On IML- 1 the samples were simply unstowed

on-orbit from passive thermal control units (PTCU's) carried in mid-deck lockers, incubated in Biorack or the Spacelab tunnel belt; and restowed in PTCUs for landing. These cultures were usc to relate the average radiation environment to mutation events..

A second hardware configuration was used to correlate specific cosmic ray strikes with specific mutations using a ray-tracing concept similar to the "Biostack" series of experiments flown on several spaceflight missions /4/. Methods were developed to immobilize nematode dauer larvae on nitocellulose filters using a 4% agarose gel overlaying technique in combination with incubation at 4° C to slow metabolism. Figure 1 illustrates the experiment configuration. Each gel/immobilized worm/filter layer was mounted onto a support and covered with two layers of CR-39 plastic nuclear track detectors. Alignment holes were drilled so that post flight disassembly would allow worm and plastic layers to be processed in parallel with coordinate matching of animals and cosmic ray tracks. Because of the practical limits to viability and fidelity of larva immobilization, fast track etching and trajectory identification procedures were developed. To maximize the likelihood of identifying "effectively hit" worms from the complete set of ion tracks, an identification algorithm was developed to select tbc higher LET particles weighted for areas with multiple hits and provided in a raster-scan file format for ease of extraction of "hit" larvae. Worms from selected coordinates were removed from the immobilized filter with a 500 μm diameter coring tool mounted via a stereotactic frame to a precision micrometer stage that in turn was matched to a system used for computerized video identification of etched tracks. The plugs of agarose with 1 or more larvae each (larval dimensions are 500 x 15 μm diameter) were inoculated onto petri dish cultures for recovery, growth and genetic analysis.

Organisms

The subjects were microscopic free-living nematodes of the species *Caenorhabditis elegans* whose biology, genetics and culture arc reviewed by Wood /5/. C. *elegans* has been studied extensively with respect to development and genetics and is notable in having a fixed cellular anatomy which has been described at the electron microscope level and whose cell lineages have been completely characterized from zygote to adult. The worm has five pairs of autosomes and 1 or 2 X chromosomes defining males (5AA + XO) and self-fertilizing hermaphrodites (5AA + XX), respectively, At 20"C a hermaphrodite will begin laying approximately 280 eggs 3 days after fertilization. Thus an 8-day spaceflight holds the potential for more than two generations, A particular larval form (dauer larva) can survive in a dormant state for several months without feeding. Dauers resume normal development approximately 12 hours after restoration of adequate food and were the sole form used in stack assemblies and the principal form used in tube cultures. Dauer larvae have approximately 12-18 germ cell precursors which arc the genetic targets and can give rise to clones of mutant progeny from single mutagenic events duc to the fact that multiple sperm and eggs may derive from a single gonia.

All strains used for IML- 1 were obtained from the *Caenorhabditis* Genetics Center at University of Missouri, Columbia, MO, (currently moving to the University of Minnesota) or were constructed in our laboratory. The wild type strain is N2 variety Bristol. The strain JP1O is a balancer chromosome strain used for isolation of lethal mutations. It has the genotype: *eT1(III;V)/dpy-18(e364)III; eT1(III;V)/unc-46(e177)V* and its phenot ypc is Wild Type with the product ion of Wild Type Uncoordinated-36 and Dumpy- 18; Uncoordinated-46 offspring in a 4:1:1 ratio.

Experimental Design

Two strategies were used for selection of mutations induced by exposure to natural space radiation. The first method used a large genetic target of 350 essential genes which arc balanced by a reciprocal translocation, *eT1 (III;V)*. This method was developed by Rosenbluth and Baillie /6/ and has been used to characterize mutagenesis by accelerated charged particles neut rons and gamma rays by Nelson and collaborators /7/. Cross section vs LET relationships for mature gametes and dauer larvae gonia have been described and provide a baseline for interpretation of space exposures. The assay measures forward autosomal lethal mutation in regions of chromosomes 3 and 5 corresponding to 15% of the worm genome or 1.2x107 base pairs of DNA. Mutants isolated in this way can be classified as to chromosomal location and type including deletion and chromosome duplication.

The second method utilizes a single large gene, uric-22, as a target. Although the target is smaller a strong selection method exists for isolation of uric-22 alleles and (heir "twitching" phenotypes arc not found associated with mutations at any other locus. The real advantage of this method is the availability of molecular probes for DNA hybridization characterization of

mutants and the extensive characterization of the chromosome region around the gene. These features arc described in /8/, A variety of *unc-22* mutants induced by accelerated particles once again serves as a baseline for comparison of IML- 1 mutants and the.ir structural spectrum vanes with particle properties /9/.

JP1O and N2 animals were inoculated into both immobilized stacks and tube cultures for isolation of lethals and twitcher mut ants.

PRELIMINARY RESULTS AND DISCUSS1ON

Based on preliminary observations there are no obvious differences in the development, behavior and chromosome mechanics of *C.elegans* as a function of gravity unloading. The animals successfully reproduced twice in space with the generation of many thousands of offspring. Both self-fertilization and mating of males with hermaphrodites was successful. Gross anatomy, symmetry and gametogenesis were normal for a small sample set based on light microscope observations. No defective karyotypes or cell distributions were observed. Finally, the pairing, disjoining and recombination of chromosomes showed no diffcrcnccs correlated with gravity levels. These results arc described in more detail in /1/. These tentative conclusions support radiobiology experiment interpretation on the basis of radiation effects without perturbation by gravity.

Physical dosimetry for each location was performed by developing TLDs and nuclear track detectors. The total TLD dose vanes from approximately 0.8 mGy in Biorack locations to 1.1 mGy in the Spacelab tunnel. Integral LET spectra from CR-39 detectors show a typical cosmic ray distribution expected for a high inclination orbit. Specifically the spectra agree well with those obtained for STS-27 and STS-28 /10/. The spectra also show an enhancement of fluence in the Spacelab tunnel over the more heavily shielded Biorack. The lower cut-off for the spectra is at 10 keV/µm and particles were classified as galactic cosmic rays if they penetrated at least four CR-39 surfaces or as short range particles if they failed to penetrate four surfaces.

Mutant isolation required a very large logistics effort to prepare and handle over 70,000 cultures for initial screening and resulted in lethal or *uric-22* mutants in each hardware component. Mutant candidates have been subjected to multiple rounds of scoring to verify heritability of defects and to verify that their properties satisfy the definitions of the mutation selections. Some strains still require verification at the time of this writing but preliminary yields arc: 12 uric-22 mutants from 1.1 million F1 animals exposed in the Spacelab tunnel, 1 uric-22 from 188000 F1's derived from immobilized dauers and 2 spontaneous mutants from 3,8 million ground control F1 's, which matches the laboratory spontaneous rate.

53 lethal mutants were isolated from flight samples and another 9 in ground controls at a rate which again matches laboratory spontaneous rates. A total of 29,700 F 1 clones were screened for lethal mutations. The frequency of flight mutants was about eight-fold above background supporting the conclusion that they were, in fact, radiation-induced. An initial classification scheme based on segregation ratios of offspring and fertility suggests that the spectra of mutants isolated randomly from tubes is qualitatively different from those correlated with specific tracks. For the latter group segregation ratios of offspring from balanced heterozygous parents were substantially different than an expected 4:1 Wild Type:Unc-36 and fertility was low. These properties arc often associated with chromosomal rearrangements. If this trend holds for IML- 1 mutants the specific track mutants should show a higher frequency of rearrangements and deletions than tube mutants. The ray-tracing method also seems to have enhanced the "capture" efficiency over random screening but this conclusion requires further analysis. Direct controls for the contribution of microgravity were not possible for track-correlated mutants as no 4° C centrifugation capability is available on Biorack. The mutant yields from matched tubes incubated on and off the centrifuge was also too low to directly evaluate a microgravity effect. However, chromosome mechanics measured by segregation and recombination were normal as was overall development; thus no obvious effects of microgravity on genetic mechanisms were detected. This indirectly argues against a contribution to mutagenesis by microgravity and other environmental factors.

In the process of screening for lethals, three morphological mutants were also isolated. These have the phenotypes: Long, Multivulva, and Roller.

SUMMARY

A variety of lethal, morphological and structural gene mutants have been isolated from nematodes flown on IML- 1 and rates of mutation were substantially above ground control spontaneous rates. Initial characterization suggests qualitative differences between mutants correlated to specific heavy ion tracks and [hose correlated only to total dose and average spectra. These

mutants will be characterized with respect to genomic structural modifications and related to the radiation field which induced them. Physical dosimctry was consistent with expected radiation fields for a shuttle mission of low altitude and high inclination.

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I-ML-I Nematode Radiation Experiment

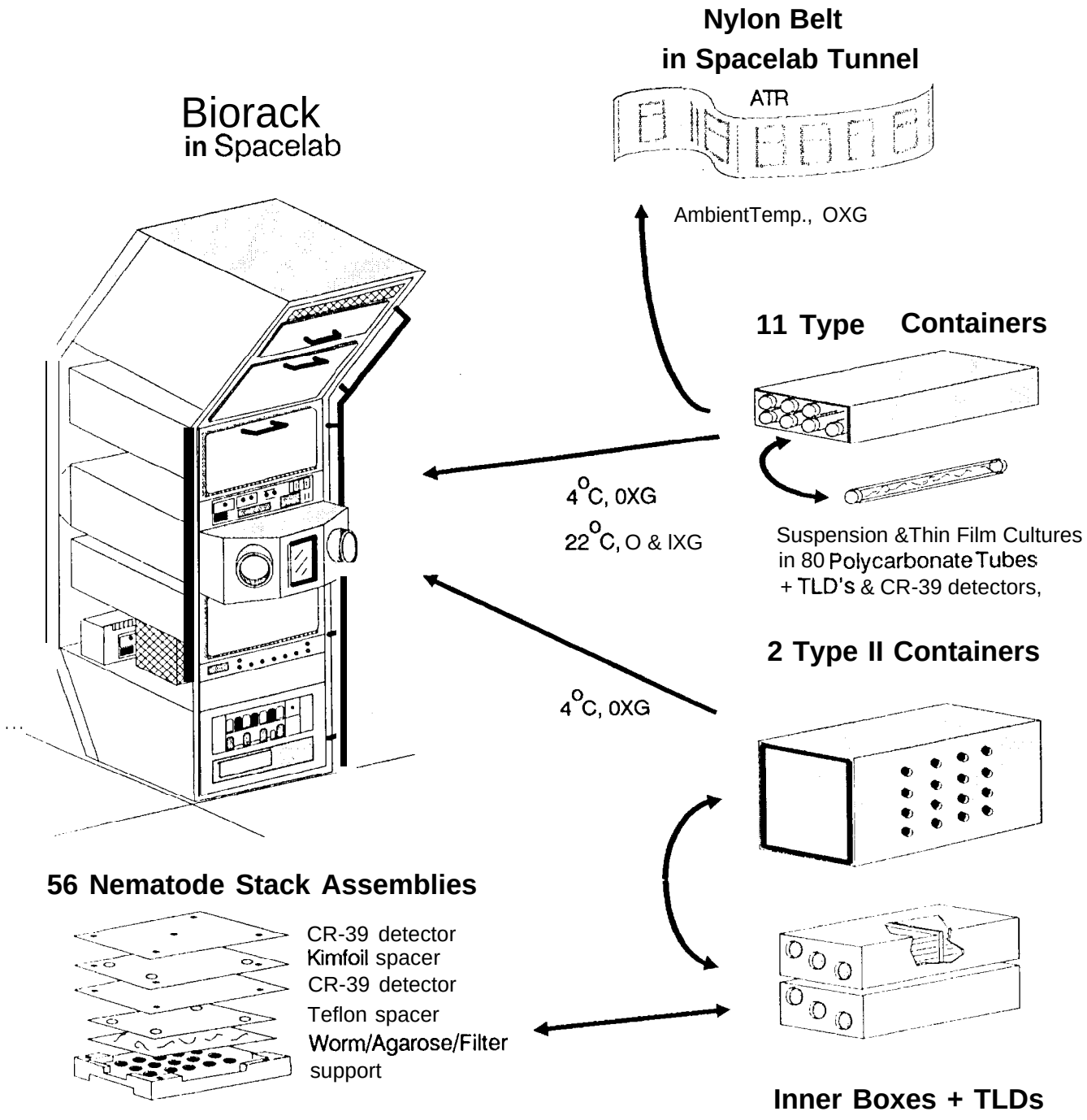

Fig. 1. The nematode radiation experiment utilized two main hardware configurations. Worms in suspension or on films of agar were cultured in lexan tubes with silicone stoppers. These were packaged in Biorack Type I containers with CR-39 nuclear track detectors and LiF thermoluminescent detectors. Type I containers were incubated in the 4°C Biorack cooler in microgravity, in the Biorack 22°C incubator in microgravity anti on the 1 XG centrifuge, and in a fabric belt placed in the Spacelab tunnel where radiation shielding is minimal. An automatic temperature recorder (ATR) monitored ambient temperature. The second hardware configuration immobilized larvae on nitrocellulose filters assembled adjacent to pairs of CR-39 nuclear track detectors in stacks so that a ray-tracing method could bc used to correlate cosmic ray tracks with individual nematodes. Nemat odc stack assembl ics were placed in Biorack Type II containers and incubatedat 4°C in the Biorack cooler.