

NEMATODE RADIOBIOLOGY AND DEVELOPMENT IN SPACE. RESULTS FROM IML-1

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The international Microgravity Laboratory 1 Spacelab mission was launched on January 22, 1997, for an 8 day mission. One of the multiuser facilities comprising the IML-1 payload was the ESA Biorack which supported 17 life sciences experiments. The experiment entitled "Genetic and Molecular Dosimetry of HZE Radiation" (mission abbreviation, *Radiat*) used Biorack and Spacelab to investigate the effects of cosmic radiation and microgravity on the stability of the genome and the fidelity of development in the small nematode, *Caenorhabditis elegans*.

The *Radiat* experiment had two objectives. The first was to isolate mutations which could be correlated to identified particle components of the space radiation environment. The second objective was to assess whether microgravity affects the development and reproduction of a small animal or the behavior of its chromosomes *in vivo*. Two different hardware designs were used to either immobilize animals next to CR-39 plastic nuclear track detectors, maintain suspensions of estimating larvae, or to facilitate growth of populations from sets of six to twelve individuals. All components were adapted to standard Biorack Type I and Type II containers and were incubated at 22° C or 4° C in Biorack or at room temperature in the Spacelab tunnel where a cloth belt held samples in this minimum radiation shielding location.

Two strategies were used for selection of mutations induced by exposure to natural space radiation. The first method used a large genetic tag of 350 essential genes which are balanced by a reciprocal translocation, *eT1(III;V)*. The assay measures forward autosomal lethal mutation in regions of chromosomes 3 and 5 corresponding to 15% of the worm genome or 1.2×10^7 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, *unc-22*, as a target. A strong selection method exists for isolation of *unc-22* alleles and their "twitching" phenotypes are not found associated with mutations at any other locus. The principal advantage of this assay is the availability of molecular probes for DNA hybridization characterization of mutants.

Physical dosimetry for each culture was performed by developing thermoluminescent detectors (TLDs) anti nuclear track detectors. The total TLD dose varied from approximately 0.8 mGy in Biorack locations to 1.1 mGy in the Spacelab tunnel. Integral LET spectra from CR-39 detectors showed a typical cosmic ray distribution expected for a high inclination orbit. The spectra also showed an enhancement of fluence in the Spacelab tunnel over the more heavily shielded Biorack.

Mutant isolation required a very large logistics effort to prepare and handle over 70,000 cultures for initial screening and resulted in lethal and *unc-22* mutants from each hardware component. Mutant candidates were subjected to multiple rounds of scoring to verify heritability of defects and to verify their phenotypes. Yields were: 12 *unc-22* mutants from F_1 animals exposed in the Spacelab tunnel, 1 *unc-22* from F_1 's derived from immobilized larvae and 2 spontaneous mutants from 3.8 million ground control F_1 's which matches the laboratory spontaneous rate. 53 lethal mutants were isolated from flight samples and another 6 arose in ground controls at a rate which matches laboratory spontaneous rates. The frequency of flight mutants was about eight-fold above background.

An initial classification scheme based on segregation ratios of offspring and fertility suggests that the spectra of mutants isolated randomly is qualitatively different from those correlated with specific tracks. The latter exhibited abnormal F_1 phenotypic ratios and low fertility which is often associated with chromosomal rearrangements. The particle spectra associated with mutants from immobilized worms was enhanced for highly ionizing particles *vis-a-vis* average spectra. In the process of *screening* for lethals, three morphological mutants were also isolated. These have the phenotypes: Long, Multivulva, and Roller. Lethal and *unc-22* mutants are currently undergoing genetic analysis to refine their structural features.

To assess chromosome mechanics at meiosis, mutant worms marked at two linked or unlinked loci were inoculated as heterozygous hermaphrodites and allowed to self fertilize. Mendelian segregation ratios and recombination frequency were measured for offspring produced at 1 \times G or in microgravity. Vigorous growth and reproduction occurred resulting in final populations of from 600 to 7320 worms per Type I container tube culture incubated at 22°C. One round of mating was efficient in space leading to many outcross progeny and a few F_2 mutant males were also present indicating that a second round of mating between F_1 animals also occurred. Thus the most complex behavior of worms appears to be insignificantly perturbed by microgravity in this hardware configuration, F_1 and F_2 progeny were produced by heterozygous hermaphrodites in phenotypic proportions which were not significantly different between controls and test samples and were representative of the expected 3:1 (plus recombinants) or 9:3:3:1 Mendelian ratios for cis-linked and unlinked loci.

To assess development, worms and embryos were fixed and stained with the DNA dye, DAPI, or antibodies specific for "P-granule" antigens (expressed in germ cells), pharyngeal myosin, body wall muscle actomyosin, and gut cells antigens. The distribution of cytoplasmic determinants, cell nuclei counts and positions were scored to assess symmetry relations and anatomical features. No obvious differences have been seen between flight and ground Wild Type animals. No defective karyotypes have been seen and the only unusual feature detected is the occasional presence of intestinal cells with incomplete nuclear divisions in both flight and ground samples. Thus development and chromosome mechanics appear to be insignificantly affected by microgravity.