1. Title

2. Research Term
FY2006～2009

3. Research Fields
Incoming Research for Space Utilization

4. Research Categories
Life Science

5. Research Theme
Establishment of novel assays for detecting transgenerational effects of space radiation using Medaka

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8. Summary of Research
During space flights, crew members are constantly exposed to space radiation. Such radiation damages the cellular DNA, and may induce mutations in germ cells. Large-scale research of the germinal mutation in the space environment has been restricted to microbes, *Caenorhabditis elegans* and *Drosophila*. Medaka (Oryzias latipes) is a useful experimental fish and precise system to measure germ cell mutation induction by specific locus test was established and found that the spontaneous and γ-ray induced mutation rates are very similar to those of mice. We are developing novel assay systems which can detect the germinal mutation using sperm genome.

Medaka is a suitable model fish for in vitro fertilization (IVF) and cryopreservation of sperm. However, the eggs or early embryos can not be cryopreserved, many females are killed to obtain unfertilized eggs for IVF. We describe a method for obtaining a large number of unfertilized eggs without killing females, using sterile interspecific hybrids between Oryzias latipes and O. curvinotus, and confirmed the reliability of the method regarding the influences on the next generation and also assessed conditions for efficient collection of unfertilized eggs (Fig. 1: Kamei et al 2007). The method would be useful to obtain large number of F1 embryos derived from male fish after long term experiment using Aquatic Habitat (AQH) in ISS and brought back to the Earth.
We established the detection system for germline mutations at microsatellite loci using medaka germ cell irradiated with ionizing radiation. To screen the mutation in medaka embryos we examined the application of high–resolution melting analysis (HRM) with 384–well formatted sample preparation. Medaka draft genome data shows 11040 microsatellite loci in LG9 and primer pairs for 5 of these microsatellite loci with high repeat number can detect radiation induced mutation in embryos derived from irradiated male germ cells.

Medaka homolog of the Drosophila vasa gene, olvas, was isolated and the transgenic medaka which allows us to monitor germ cells by GFP fluorescence was established (Tanaka et al. 2001). The transgenic strain allows us to examine the radiation effects on medaka germ cells in live specimens. Sperms of this strain are labelled with GFP and this transgene could be used for specific–locus test system (SLT) using medaka sperm.

Transgenic strains with olvas promoter regulated gene for expressing YFP with signal sequences for mitochondrial translocation and a gene for expressing mCherry are establishing to establish SLT test at multiple loci. And we also examined the bacterial tetracycline operator/repressor (TetO/TetR) system in cultured medaka cell line to apply SLT. TerR can suppress TetO dependent GFP expression in medaka cells. But basal GFP level in TetO/TetR cells are transiently increased by irradiation, so we have to determine the reliable time interval between irradiation and assay to determine the mutation frequency

9. Publication List

10. URL