Published in final edited form as:
Biol Sci Space. 2009 October 1; 23(4): 183–187. doi:10.2187/bss.23.183.

C. elegans RNAi space experiment (CERISE) in Japanese Experiment Module KIBO

Atsushi Higashitani[1,*], **Toko Hashizume**[2], **Tomoko Sugimoto**[3], **Chihiro Mori**[1], **Kanako Nemoto**[1], **Timothy Etheridge**[4], **Nahoko Higashitani**[1], **Takako Takanami**[1], **Hiromi Suzuki**[5], **Keiji Fukui**[5], **Takashi Yamazaki**[3], **Noriaki Ishioka**[3], **Nathaniel Szewczyk**[4,*], and **Akira Higashibata**[3,*]

[1]Graduate School of Life Sciences, Tohoku University, Sendai, 980-8577, Japan [2]Advanced Engineering Services Co., Ltd., Tsukuba, 305-0032, Japan [3]ISS Science Project Office, Institute of Space and Astronautical Science, Japan Aerospace Exploration Agency, Tsukuba, 305-8505, Japan [4]University of Nottingham, Royal Derby Hospital, Uttoxeter New Road, DE22 3DT, England [5]Japan Space Forum, Ohtemachi, Chiyoda-ku, 100-0004, Japan

Abstract

We have started a space experiment using an experimental organism, the nematode Caenorhabditis elegans, in the Japanese Experiment Module, KIBO, of the International Space Station (ISS). The specimens were boarded by space shuttle Atlantis on mission STS-129 which launched from NASA Kennedy Space Center on November 16, 2009. The purpose of the experiment was several-fold: (i) to verify the efficacy of RNA interference (RNAi) in space, (ii) to monitor transcriptional and post-translational alterations in the entire genome in space, and (iii) to investigate mechanisms regulating and countermeasures for muscle alterations in response to the space environment. In particular, this will be the first study to utilize RNAi in space.

Introduction

Caenorhabditis elegans (*C. elegans*) is a free-living, non-parasitic soil nematode. It can be easily manipulated, observed and cultivated in the laboratory owing to its small size (an adult worm is approximately 1 mm in length), transparency and feeding on bacteria. A wealth of studies over the past few decades have resulted in *C. elegans* becoming a well-known model organism. For example, the complete cell-lineage, neuronal networks, muscle anatomy and genome sequence, make this an excellent in vivo model in which to conduct biological research both on Earth and in space. The first trials on the effects of the space environment, including cosmic radiations, upon *C. elegans* were performed using essentially standard culturing techniques (Johnson and Nelson, 1991; Nelson *et al*., 1994a,b; Hartman *et al*., 2001). Subsequently, a complete chemical liquid medium (CeMM) for use with *C. elegans* was prepared by Szewczyk et. al., (2003, 2006), which allowed study of the effects of surface tension in flight. During the Dutch Soyuz mission DELTA to the ISS in April 2004, an international collaboration of laboratories carried out the “FIRST International *C. elegans* Experiment in space” (ICE FIRST). One of the main goals of this experiment was to validate the biological response of *C. elegans* to 10 day spaceflight. Consistent with past

[*]To whom correspondence should be addressed: A. Higashitani: ahigashi@ige.tohoku.ac.jp; N. Szewczyk: Nathaniel.Szewczyk@nottingham.ac.uk; A. Higashibata: higashibata.akira@jaxa.jp.

experiments, animals displayed a normal rate of development in flight and returned in good apparent health. With the exception of a slight movement defect upon return to Earth, which appears to be due to altered muscle development in flight (Higashibata *et al*., 2006), no significant abnormalities were detected. Apoptosis also proceeded normally (Higashitani *et al*., 2005) and the rate of mutation associated with flight was below the experiments limits of detection (Zhao *et al*., 2006). These results appear similar to what is observed for humans and suggest that *C. elegans* can be used to study responses to spaceflight and may be developed as a biological sensor (Zhao *et al*., 2005; Custodia *et al*., 2001). At the molecular level, transcriptome and proteome analyses indicated decreased expression of muscle related genes and proteins, respectively, including altered expression of certain genes regulated by insulin and transforming growth factor-β (TGF-β) signalling in response to spaceflight (Higashibata *et al*., 2006, 2007, Selch *et al*., 2008).

C. elegans is the first animal in which RNA interference (RNAi) by double stranded RNA (dsRNA) was observed (Fire *et al*., 1998). RNAi is an evolutionarily conserved mechanism for silencing gene expression (Novina and Sharp 2004, Tomari and Zamore 2005). RNAi protects the genome from viruses and other insertable genetic elements and regulates gene expression during development. The antisense strand derived from the dsRNAs incorporates into an RNA-induced silencing complex that can either direct degradation of target mRNA or suppress the protein(s) it encodes from being expressed (Novina and Sharp 2004, Tomari and Zamore 2005). The discovery that RNAi works in mammalian cells has sparked intense investigation into its role in normal mammalian cell function, its use as a tool to understand or screen for genes functioning in cellular pathways in healthy and diseased cells and animals, and its potential for therapeutic gene silencing (Dykxhoorn and Lieberman 2005, Aigner 2006, Chakraborty 2007, DeVincenzo 2009). The ensuing results suggest RNAi may provide an important new therapeutic modality for treating infection, cancer, neurodegenerative disease, and other illnesses.

Here we introduce our current space experiment termed CERISE (*C. elegans* RNA interference Space Experiment) in the Japanese Experiment Module (JEM), called KIBO, on the International Space Station (ISS) beginning November 2009. A proposal for CERISE was reviewed and accepted by the International Space Life Sciences Working Group in 2004; the CERISE decal is shown in Fig. 1. The experimental aims are to verify the efficacy of RNAi in space and to analyze changes in the transcriptome and proteome in response to the space environment. We further aim to investigate mechanisms of and countermeasures for musclular alterations in response to spaceflight.

Experimental Design

Nematode eggs were prepared using the alkaline bleach method with 0.5N KOH and 1.0% NaClO. After overnight incubation in M9 buffer containing 5mg/ L cholesterol at 20° C, the hatched L1 larvae were used for the space experiment. For most experiments, approximately 9,000 L1 were used for samples to be collected 4 days post-acitvation and 30 to 50 L1 were used for samples to be collected 8 days post-activation. For the muscle protein degradation studies, approximately 1,000 dauers, prepared according to the protocol of Hartman *et al*., (2001) with the exception that animals were cultured on 8X peptone NGM agar plates, were used with sample collection on day 4 post-activation. All larvae were maintained in the left compartment of the culturing bags in 2 ml S basal medium (Fig. 2). The right compartment of the culturing bag system, separated by a U-pin, contained bacterial feeds in 12 ml S basal medium at an OD600 of approximately 3.5. Bags were then stored in the Meas Exp. Unit A (15 bags per unit, Fig. 2). On 16th November 2009, Shuttle Atlantis (STS-129, ULF-3) launched from Kennedy Space Center, Florida. Upon reaching microgravity at KIBO on the ISS, flight crews activated the experiments by removing the U-pin (19th November), at

which time four Meas Exp. Unit A were transferred into the Cell Biology Experiment Facility (CBEF) with or without 1 *G* rotation for either 4 or 8 days. After observation of the nematodes by microscopy, experiments were stopped by freezing and subsequent storage at −80° C in MELFI. For the 4 day culture experiments, the L1 or dauer larva grew to adulthood as the first generation, and for the 8 day experiments the L1 larvae's second generations grew to adulthood under exposure to space environment from fertilization.

RNA interferences (RNAi)

To evaluate the efficacy of RNAi in the space environment, we used two target genes, *Cerbx-1* (Sasagawa *et al*., 2003) and recombinant green fluorescent protein (GFP) genes. Strains AZ212 (ruls32; *unc-119* (*ed3*), Praitis *et al*., 2001), whose integrated array is pAZ132 (*pie-1::GFP::histone H2B* fusion and *unc-119* subclone), and PD4251 (ccls4251; *dpy-20* (*e1282*), Fire *et al*., 1998), whose integrated array contains three plasmids: pSAK2 (*myo-3* promoter driving a nuclear-targeted DFP-LacZ fusion), pSAK4 (myo-3 promoter driving mitochondrial-targeted GFP), and a *dpy-20* subclone were used in this experiment. AZ212 GFP signals in the nuclei of oocytes and eggs, and PD4251 fluoresces in the nuclei and mitochondria of body wall muscles (Fig. 3). Double stranded RNA of *Cerbx-1* and *gfp* genes were synthesized in *Escherichia coli* HT115 (DE3) with Litmus 28 plasmid vector *in vivo* system (Sasagawa *et al*., 2003). Following experiment activation and thus introduction of the nematodes to the bacteria, animals fed on the bacteria (termed feeding RNAi, Kamath *et al*., 2000) in the space environment. In addition, we have performed feeding RNAi against both *asp-4* and *asp-6*, genes that encode aspartyl proteases, in order to study the effects of depletion of aspartyl protease on muscle protein degradation in response to microgravity.

Transcriptome and Proteome analyses

To study the molecules and signals that are affected in response to the space environment, we have carried out transcriptome analyses using full genome DNA microarrays and proteome analyses, including post transcriptional modification. We have previously shown that both expression of the transcription factors for myosin heavy chains (MHC) and MHC genes themselves are down-regulated in response to spaceflight (Higashibata *et al*., 2006). Insulin and TGF-β regulated genes also displayed alterations (Selch *et al*., 2008). Additionally, proteomic analysis using 2-dimensional gel electrophoresis indicated that approximately 10-15% of spots (i.e. detectable proteins in the proteome) significantly increased or decreased in the flight samples compared with the ground control (Higashibata *et al*., 2007). While much information on the transcriptional and translational adaptations to spaceflight was gained from ICE FIRST, there were some experimental limitations such as mixed stage samples (non-synchronized culture) and late fixation (freezing after return to Earth). In the present experiments we, therefore, aim to confirm reproducibility of the above results by using a synchronized culturing system with on orbit fixation. We shall also perform comparative analysis of phosphorylated proteins between culturing at microgravity versus a 1 *G* control using defective mutants of stress activated MAPK p38 and JNK1(strains KU25: *pmk-1* (*km25*) and VC8: *jnk-1* (*gk7*)).

Muscle synthesis and degradation in space

It is well-known that the neuromuscular system is one of the physiologic systems most affected by spaceflight (Fitts *et al*., 2001). Muscles developed on Earth alter in morphology, contractile function and MHC gene expression during spaceflight, or unloading on Earth (Caiozzo *et al*., 1994; 1996; Criswell *et al*., 1996; Day *et al*., 1995; Harrison *et al*., 2003). In addition to depressed de novo synthesis, specific protein degradation systems, such as ubiquitin ligase(s)-mediated proteasomal degradation have been shown to be involved in skeletal muscle atrophy (Bodine *et al*., 2001; Gomes *et al*., 2001). For example, in denervated muscle of rats, mRNA levels of MHC I are decreased and mRNA levels of

atrogin-1 (a ubiquitin ligase gene) are significantly increased (Horinouchi *et al*., 2005). However, the relative contributions of specific molecular changes within muscle to more global changes in muscle remains an area of active research. In *C. elegans*, we are studying the signal-transduction networks regulating muscle protein degradation (Szewczyk and Jacobson 2005, Szewczyk *et al*., 2007). At present we have demonstrated the existence of three distinct regulatory networks in *C. elegans* muscle. First, proteasome based degradation appears to be regulated by a molecular network tied to muscle cell depolarization. Second, autophagic based degradation appears to be regulated by a molecular network tied to growth factor signaling. Third, an unknown protease is regulated by a molecular network tied to muscle attachment to the extracellular matrix. In this flight experiment, investigations of both muscle development and atrophy in *C. elegans* are performed. We will utilize our standard methods to assess cytosolic muscle protein degradation in wild-type animals and in animals with blocked proteasomal degradation (MG132 inhibited) or blocked autophagic degradation (*asp-4* and *asp-6* RNAi). Additionally, as caspases are well known proteases and mutants for *ced-3* are readily available, we will also examine cytosolic protein degradation in *ced-3* mutant animals. Lastly, we have also included experiments that applied methyl-cellulose to the culture media to increase viscosity to understand whether increasing viscosity upregulates *de novo* muscle synthesis and / or represses muscle degradation in *C. elegans*.

Conclusion

We anticipate that the analyses of CERISE flight samples will provide us information on the physiological and molecular biological effects of microgravity / spaceflight on the experimental model *C. elegans*. In addition, it is essential to verify the efficacy of RNAi for its subsequent application in both future basic science and applied clinical use in the space environment. These samples have already been successfully launched, cultured and stored in MELFI Deep Freezer of ISS, and will be returned by Space Shuttle Endeavor (STS-130) in February, 2009.

Acknowledgments

The CERISE experiment has been conducted in the Japanese Experimental Module “Kibo” of the International Space Station. We have to thank crew members, lots of scientists and coordinators of JAXA and NASA for kindly and helpfully supporting our experiment. We also thank the Caenorhabditis elegans Genetic Center for the kind supply of mutant and recombinant strains. This work is supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and from the Japan Society for the Promotion of Science (JSPS). This study is also performed as part of the “Ground-Based Research Announcement for Space Utilization,” promoted by the Japan Space Forum. In addition, CM is supported by the International Advanced Research and Education Organization in Tohoku University. TE and NS’ participation are supported by the UK MRC (G0801271) and the US NIH NIAMS (AR054342).

References

Aigner A. Gene silencing through RNA interference (RNAi) in vivo: Strategies based on the direct application of siRNAs. J Biotechnol 2006;124:12–25. [PubMed: 16413079]

Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, et al. Identification of ubiquitin ligases required for skeletal muscle atrophy. Science 2001;294:1704–1708. [PubMed: 11679633]

Caiozzo VJ, Baker MJ, Herrick RE, Tao M, Baldwin KM. Effect of spaceflight on skeletal muscle: mechanical properties and myosin isoform content of a slow muscle. J Appl Physiol 1994;76:1764–1773. [PubMed: 8045858]

Caiozzo VJ, Haddad F, Baker MJ, Herrick RE, Prietto N, Baldwin KM. Microgravity-induced transformations of myosin isoforms and contractile properties of skeletal muscle. J Appl Physiol 1996;81:123–132. [PubMed: 8828654]

Chakraborty C. Potentiality of small interfering RNAs (siRNA) as recent therapeutic targets for gene-silencing. Curr Drug Tartget 2007;8:469–482.

Criswell DS, Carson JA, Booth FW. Regulation of contractile protein gene expression in unloaded mouse skeletal muscle. J Gravit Physiol 1996;3:58–60. [PubMed: 11540283]

Custodia N, Won SJ, Novillo A, Wieland M, Li C, Callard IP. Caenorhabditis elegans as an environmental monitor using DNA microarray analysis. Ann N Y Acad Sci 2001;948:32–42. [PubMed: 11795393]

Day MK, Allen DL, Mohajerani L, Greenisen MC, Roy RR, Edgerton VR. Adaptations of human skeletal muscle fibers to spaceflight. J Gravit Physiol 1995;2:47–50.

DeVincenzo JP. Harnessing RNA interference to develop neonatal therapies: From Nobel prize winning discovery to proof of concept clinical trials. Early Human Dev 2009;85:S31–S35.

Dykxhoorn DM, Lieberman J. The silent revolution: RNA interference as basic biology, research tool, and therapeutic. Ann Rev Med 2005;56:401–423. [PubMed: 15660519]

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 1998;391:806–811. [PubMed: 9486653]

Fitts RH, Riley DR, Widrick JJ. Functional and structural adaptations of skeletal muscle to microgravity. J Exp Biol 2001;204:3201–3208. [PubMed: 11581335]

Gomes MD, Lecker SH, Jagoe RT, Navon A, Goldberg AL. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. Proc Natl Acad Sci U S A 2001;98:14440–14445. [PubMed: 11717410]

Harrison BC, Allen DL, Girten B, Stodieck LS, Kostenuik PJ, Bateman TA, Morony S, Lacey D, Leinwand LA. Skeletal muscle adaptations to microgravity exposure in the mouse. J Appl Physiol 2003;95:2462–2470. [PubMed: 12882990]

Hartman PS, Hlavacek A, Wilde H, Lewicki D, Schubert W, Kern RG, Kazarians GA, Benton EV, Benton ER, Nelson GA. A comparison of mutations induced by accelerated iron particles versus those induced by low earth orbit space radiation in the FEM-3 gene of Caenorhabditis elegans. Mutat Res 2001;474:47–55. [PubMed: 11239962]

Higashibata A, Szewczyk NJ, Conley CA, Imamizo-Sato M, Higashitani A, Ishioka N. Decreased expression of myogenic transcription factors and myosin heavy chains in Caenorhabditis elegans muscles developed during spaceflight. J Exp Biol 2006;209:3209–3218. [PubMed: 16888068]

Higashibata A, Higashitani A, Adachi R, Kagawa H, Honda S, Honda Y, Higashitani N, Sasagawa Y, Miyazawa Y, Szewczyk NJ, Conley CA, Fujimoto N, Fukui K, Shimazu T, Kuriyama K, Ishioka N. Biochemical and Molecular Biological Analyses of space-flown nematodes in Japan, the First International Caenorhabditis elegans Experiment (ICE-First). Microgravity Sci Technol 2007;19:159–163. [PubMed: 19513185]

Higashitani A, Higashibata A, Sasagawa Y, Sugimoto T, Miyazawa Y, Szewczyk NJ, Viso M, Gasset G, Eche B, Fukui K, Shimazu T, Fujimoto N, Kuriyama K, Ishioka N. Checkpoint and physiological apoptosis in germ cells proceeds normally in spaceflown *Caenorhabditis elegans*. Apoptosis 2005;10:949–954. [PubMed: 16151630]

Horinouchi H, Kumamoto T, Kimura N, Ueyama H, Tsuda T. Myosin loss in denervated rat soleus muscle after dexamethasone treatment. Pathobiology 2005;72:108–116. [PubMed: 15860927]

Johnson TE, Nelson GA. Caenorhabditis elegans: a model system for space biology studies. Exp Gerontol 1991;26:299–309. [PubMed: 1915699]

Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG, Ahringer J. Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. Genome Biol 2000;2:research 0002.1–0002.10.

Nelson GA, Schubert WW, Kazarians GA, Richards GF. Development and chromosome mechanics in nematodes: results from IML-1. Adv Space Res 1994a;14:209–214. [PubMed: 11537919]

Nelson GA, Schubert WW, Kazarians GA, Richards GF, Benton EV, Benton ER, Henke R. Radiation effects in nematodes: results from IML-1 experiments. Adv Space Res 1994b;14:87–91. [PubMed: 11540032]

Novina CD, Sharp PA. The RNAi revolution. Nature 2004;430:161–164. [PubMed: 15241403]

Praitis V, Casey E, Collar D, Austin J. Creation of low-copy integrated transgenic lines in *Caenorhabditis elegans*. Genetics 2001;157:1217–1226. [PubMed: 11238406]

Sasagawa Y, Uranno T, Kohara Y, Takahashi H, Higashitani A. Caenorhabdits elegans RBX1 is essential for meiosis, mitotic chromosomal condensation and segregation, and cytokinesis. Genes Cells 2003;8:857–872. [PubMed: 14622138]

Selch F, Higashibata A, Imamizo-Sato M, Higashitani A, Ishioka N, Szewczyk NJ, Conley CA. Genomic response of the nematode Caenorhabditis elegans to spaceflight. Adv Space Res 2008;41:807–815. [PubMed: 18392117]

Szewczyk NJ, Kozak E, Conley CA. Chemically defined medium and *Caenorhabditis elegans*. BMC Biotechnol 2003;3:19, 1–7. [PubMed: 14580264]

Szewczyk NJ, Peterson BK, Barmada SJ, Parkinson LP, Jacobson LA. Opposed growth factor signals control protein degradation in muscles of *Caenorhabditis elegans*. EMBO J 2007;26:935–943. [PubMed: 17290229]

Tomari Y, Zamore PD. Perspective: machines for RNAi. Genes Dev 2005;19:517–529. [PubMed: 15741316]

Zhao Y, Johnsen R, Baillie D, Rose A. Worms in space? A model biological dosimeter. Gravit Space Biol Bull 2005;18:11–16. [PubMed: 16038089]

Zhao Y, Lai K, Cheung I, Youds J, Tarailo M, Tarailo S, Rose A. A mutational analysis of Caenorhabditis elegans in space. Mutat Res 2006;601:19–29. [PubMed: 16765996]

Fig. 1.
Decal for the CERISE experiment

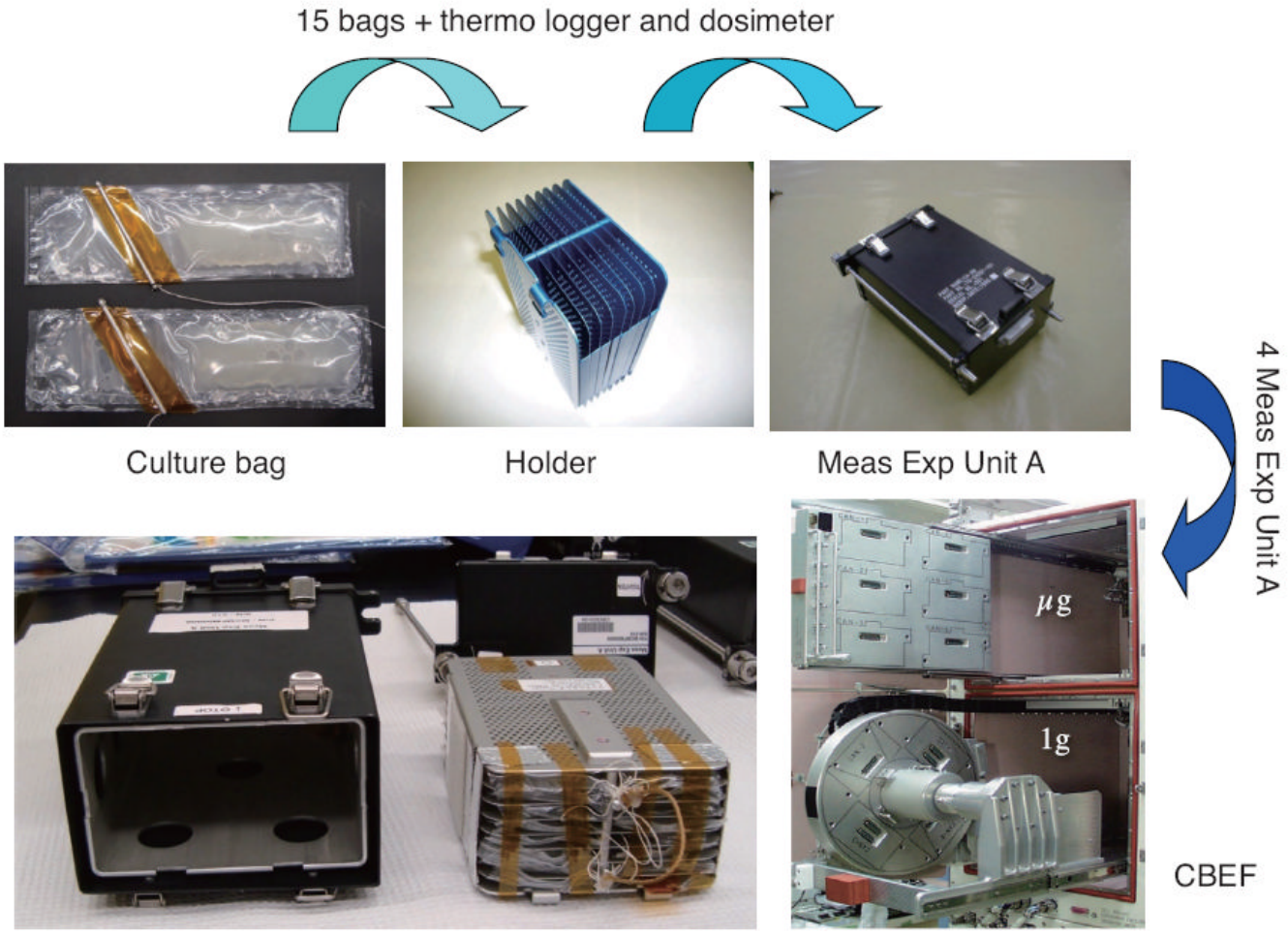

Fig. 2.

Experimental equipments and culture bags. The bags were made of polyethylene with heat shielding. Two compartments were separated with U-pin. Crew member removes the U-pin in space, and cultures are started in CBEF.

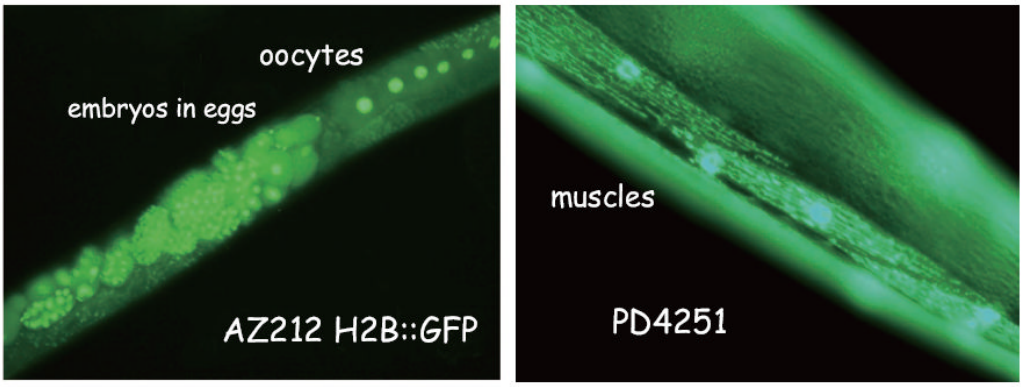

Fig. 3.
GFP recombinant strains used in this study to monitor RNAi activity and muscle structures. Nuclei of Oocytes and embryos are visualized in AZ212 (left panel). Nuclei and mitochondria of muscles are visualized in PD4251 (Right panel).