Report of HIMAC experiments granted by "Living in Space"

(A03-1) "Multidisciplinary Analysis of the Effect of Low Fluence Particle Radiation on Animals and Biological Adaptations"
Research Group Leader: Mitsuru Nenoi
Visit duration: 24th of January to 2nd of February,2018

Experiment / project name: Visualization of the DNA strand break repair by non-homologous end joining and homologous recombination in low and high LET irradiated *Bacillus subtilis* spores (insight-REPAIR) [HIMAC Project no. 15J410]

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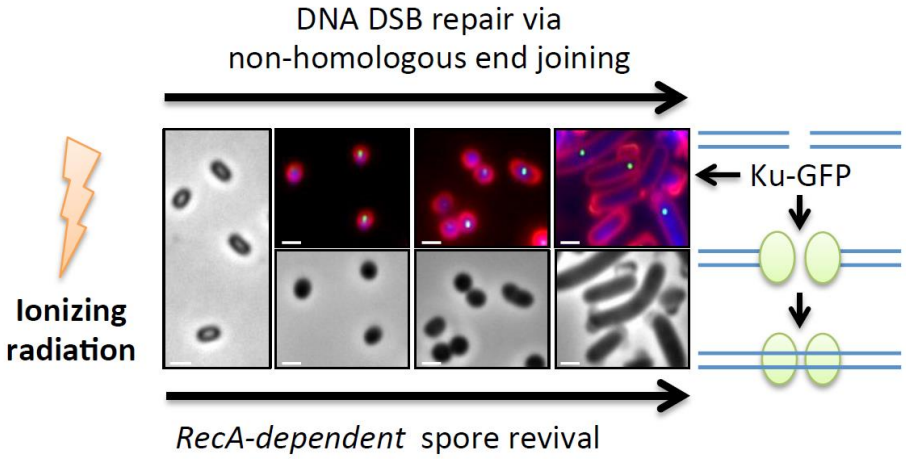
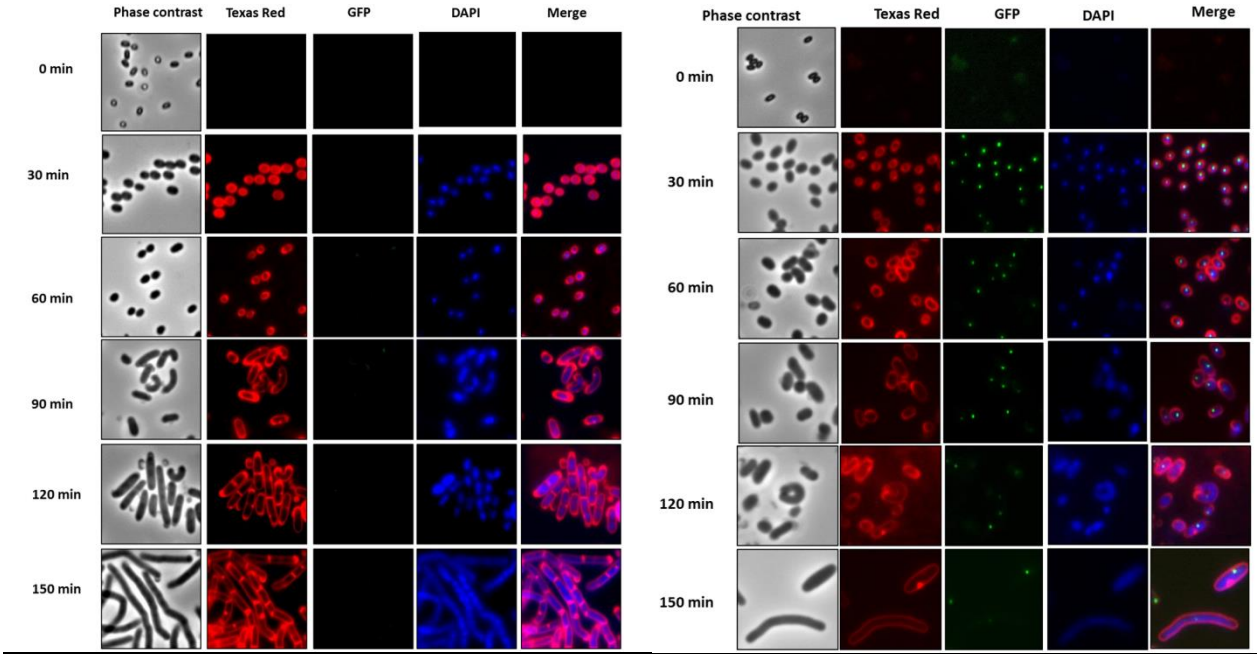
Beam time (date and heavy ions):
1) January 26th, 2018 (22:00 – 30:00 (10:00 p.m. – 06:00 a.m.)), Argon Ar 500 MeV/n (LET 90 keV/µm)
2) January 28th, 2018 (22:00 – 28:00 (10:00 p.m. – 04:00 a.m.)), Helium He 150 MeV/n (LET 2 keV/µm)
3) January 30th, 2018 (22:00 – 30:00 (10:00 p.m. – 06:00 a.m.)), Iron Fe 500 MeV/n (LET 200 keV/µm)

Aim and scope of experiment: *In-depth knowledge regarding the biological effects of the radiation field in space* is required for assessing radiation risks to humans in space. To obtain this knowledge, microorganisms, plants, and animals have been studied as radiobiological model systems in space and at heavy ion accelerators on the ground. In a variety of space experiments, *spores of Bacillus subtilis* have been used as *biological dosimeters* at the µm scale to determine radial biological efficiency along the trajectories of individual HZE particles. *DNA double-strand breaks (DSBs) are the most severe type of damage* induced by HZE particles in microorganisms. Microorganisms possess several mechanisms to repair DNA DSBs induced by HZE particles. Because high energy charged particles (HZE) particles are considered as important components among the cosmic rays, causing high levels of radiobiological damage, studies on biological effects of HZE particles of GCR in space as well as of accelerated heavy ions at ground based facilities are useful for the estimation of the biological influences of space radiation environments. In insight-REPAIR it is aimed to gain *new insights in activity, speed and accuracy of non-homologous end joining (NHEJ)- and RecA-mediated recombinational repair* during the germination in time-lapsed manner using fluorescence microscopy (e.g., foci kinetic analysis).



Visualization of the DNA strand break repair in germinating and outgrowing spores of *B. subtilis*.



Activity of non-homologous end joining (NHEJ)-mediated repair using fluorescent microscopy.

Highlight results: Spore survival data altogether suggest that both Ku and RecA play an active role in spore recovery after irradiation with He or Fe ions, but how these processes are regulated is poorly understood. To gain insight into the mechanisms that influence the selection of NHEJ and how this process is regulated during spore revival, the fates of Ku-GFP (initial player in NHEJ) and RecA-YFP (central player in HR) were studied upon spore revival. To study the role of Ku and RecA in the presence of pre-existing DSB, mature spores were exposed to heavy ion radiation, and upon synchronizing revival time-course microscopy was employed to track Ku-GFP or RecA-YFP variants. After irradiation mature ku-gfp spores showed survival curves similar to wild-type, indicating the presence of a functional protein fusion. Ku-GFP foci were located in the center of the spore core, where the DNA is found.

Upon exposure to an array of molecules that trigger germination, the spore undergoes rehydration. A characteristic step ensuing with spore rehydration is the transition from a phase-bright to a phase-dark spore, which can be observed by phase contrast microscopy. After the phase-transition, no morphological change was evident 60 min into spore revival (germination and ripening period), but at 90 min into revival, cell and nucleoid size increased (outgrowth period). Ku-GFP foci formation was observed in spores treated with all tested doses of He ions and increased dose-dependently. When mature spores were exposed to a dose of 100 Gy, which only reduced ~20% spore viability, Ku-GFP formed a discrete focus in ~27% of ripening spores (30 min). The number of DSBs when mature spores were exposed to 100 Gy of He ion radiation is unknown. Ku-GFP foci formation peaked at 60 min (~43%) to decrease at later times (bell-shaped curve), suggesting that Ku activity and/or recruitment to DSBs was positively (during ripening) and negatively (during outgrowth and transition to vegetative cells) regulated by unknown (a) factor(s).

Here, we propose that NHEJ is favored for DSB repair in the absence of replication, provided that Ku levels are high. By contrast, low Ku and increased RecA levels contribute to recombination-dependent DNA replication. Additional work on the precise level of the heavy ion irradiation-induced spore DNA damage is required to obtain a complete understanding on spore resistance to space radiation.

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