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:18th to 28th of June 2018

Experiment / project name: Determination of the collaborative efforts and interaction of different mechanisms in the DNA repair of low and high LET radiation induced damage in Bacillus subtilis spores (CO-REPAIR) [HIMAC Project no. 17J422]

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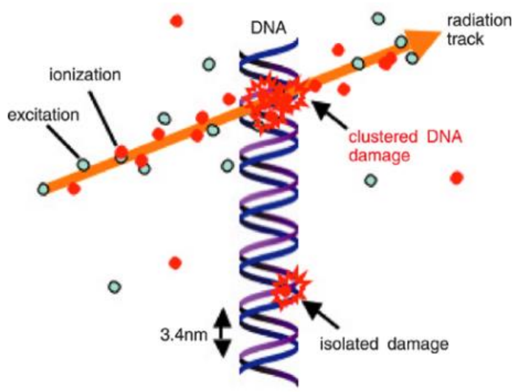
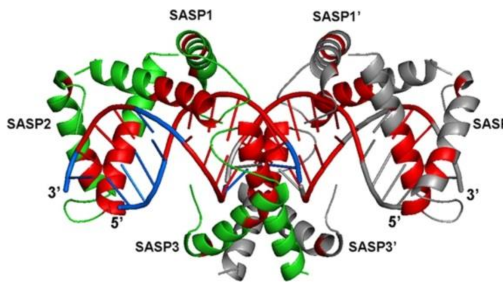
Beam time (date and heavy ions):
1) June 21th, 2018 (22:00 – 30:00 (10:00 p.m. – 06:00 a.m.)), Helium He 150 MeV/n (LET 2 keV/µm)
2) June 25th, 2018 (22:00 – 30:00 (10:00 p.m. – 06:00 a.m.)), Iron Fe 500 MeV/n (LET 200 keV/µm)

Aim and scope of experiment: All terrestrial organisms have evolved mechanisms to maintain genomic integrity in the face of extreme environmental stresses. One class of microorganisms (the so-called extremophiles) is known to survive extreme environmental (and even extraterrestrial) conditions including of high doses of ionizing radiation. The results reinforced the notion that survival after high doses of ionizing radiation does not depend on a single mechanism or process, but instead is multifaceted. Many identified genes affect either DNA repair or the cellular response to oxidative damage. However, contributions by secondary and even tertiary mechanisms or pathways involved in other key physiological processes are also evident.

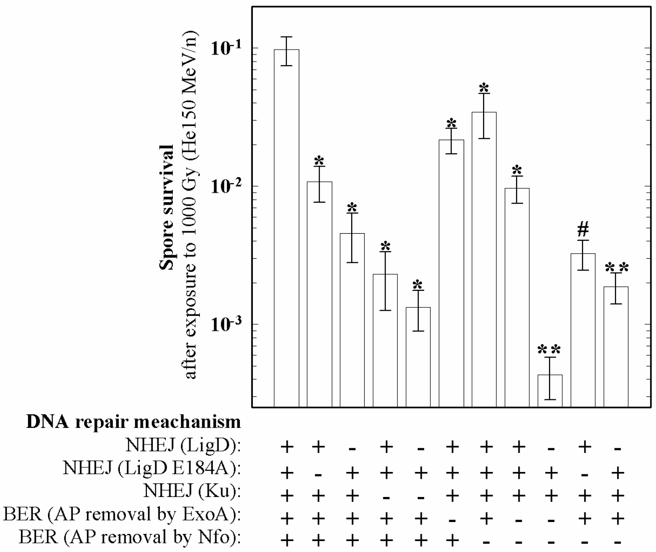




DNA is damaged due to ionization or excitation caused by high LET radiation.

Within the research project CO-REPAIR is aimed to study the several DNA repair mechanisms (e.g., spore photoproduct lyase (SP lyase), nucleotide excision repair (NER), base excision repair (BER), recombinational repair (HR), DNA integrity scanning protein (DisA), phosphodiesterase activity, translesion synthesis (TLS), transcription-coupled repair (TCR), interstrand cross-link (ICL) repair), mismatch repair, MMR) as single knockout cell lines as combined with an additional mutation in

NHEJ. A major focus will lay on the identification of the collaborative and supporting efforts of other DNA repair mechanisms in the process of NHEJ as the major DNA double strand break (DSB) repair pathway. With this project, spores of Bacillus subtilis 168 (from wild-type and selected knockout mutants) will be used to systematically analyze their impact in spore survival, germination and outgrowth behavior (e.g., DPA release) and repair kinetics (e.g., DNA DSB repair).
Highlight results: Our first preliminary data show that DNA repair mutant spores, lacking in both non-homologous end joining (NHEJ) and base excision repair (BER), are more sensitive to He particle radiation than the respective single (NHEJ or BER) repair-deficient strain.



Spore survival of LigD E184A (catalytic residues responsible for the ligation activity of LigD in NHEJ), ΔLigD (DNA repair polymerase/ligase, NHEJ), ΔKu (DNA-end-binding protein, NHEJ), ΔExoA (apurinic / apyrimidinic endonuclease, BER), ΔNfo (spore-specific AP endonuclease IV, BER) deficient strains after exposure to 1000 Gy of He particle irradiation (150 MeV/n, LET: 2.2 keV/µm). * significant different than the wild-type, ** significant different than the ΔLigD and ΔExoA-Nfo strains, # significant different than the LigD E184A strain (P value of 0.05).

The absence of ExoA and/or Nfo in deletion mutant strains of B. subtilis sensitized the spores to He radiation that damage spore DNA through generation of AP sites and strand breaks, suggesting that BER should be active to repair the lesions during spore germination and outgrowth that have accumulated during spore dormancy. The action of these AP endonucleases on AP sites renders a gap; accomplishment of AP site repair would require a polymerization step to close the gap with a ligase activity to seal the final nick. Here LigD could potentially participate in BER since this enzyme has shown to efficiently fill a single nucleotide gap on AP-DNA. The ligation functions of LigD could be necessary and sufficient for 'short patch' BER of AP sites during spore germination and outgrowth together with the B. subtilis AP endonucleases Nfo and/or ExoA. Therefore, the bacterial LigD complex had been exclusively involved in the repair of DSB through the NHEJ pathway; the results presented here are suggestive of a potential participation of this protein in bacterial BER as well. Altogether, our first results suggest that the role of the ATP-dependent ligase domain is not restricted to the final strand closure after HZE particle irradiation and paving the way to future works aimed to decipher the interplay with other DNA repair proteins of the NHEJ and/or BER pathway after irradiation. The research project CO-REPAIR is designed to provide new insights in interaction and teamwork on spore-specific and universal DNA repair mechanisms. Previous studies have indicated that in both pro- and eukaryotic cells or under highly scavenging conditions mimicking those for ROS scavenging in the cell, one fourth of the lesions induced in DNA by low LET radiation can be ascribed to direct effects increasing up to 80% for high LET particles. Lethal and mutagenic effects induced by ionizing radiation are thought to be the result of DNA damage caused during the course of irradiation. Spore DNA resides in the innermost spore compartment, the core, and dormant spores of B. subtilis possess a complex arsenal of protective attributes in the core. Spore DNA damage can also be repaired during germination via a number of different pathways; however there has been no systematic study of the relationship and "teamwork" between different DNA repair mechanisms in spores' resistance to low and high LET radiation.

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