BYSTANDER EFFECT AFTER EXPOSURE TO HIGH-LET PARTICLES ENCOUNTERED IN COSMIC RADIATION

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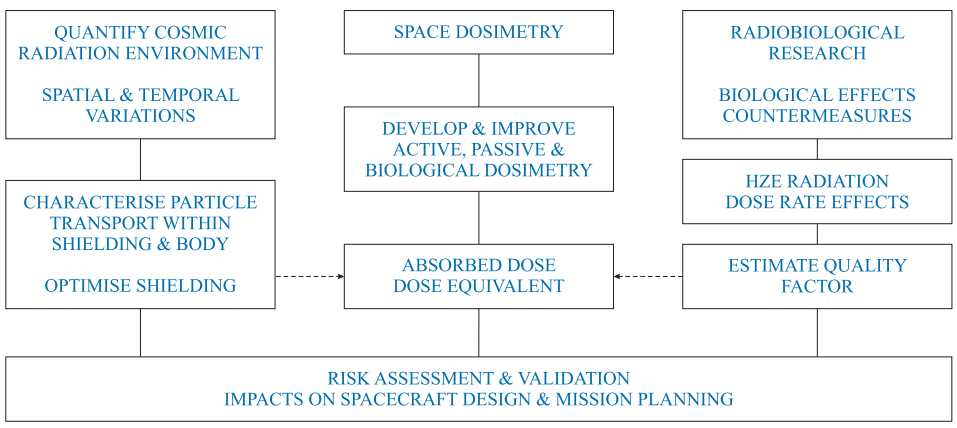
INTRODUCTION & MOTIVATION

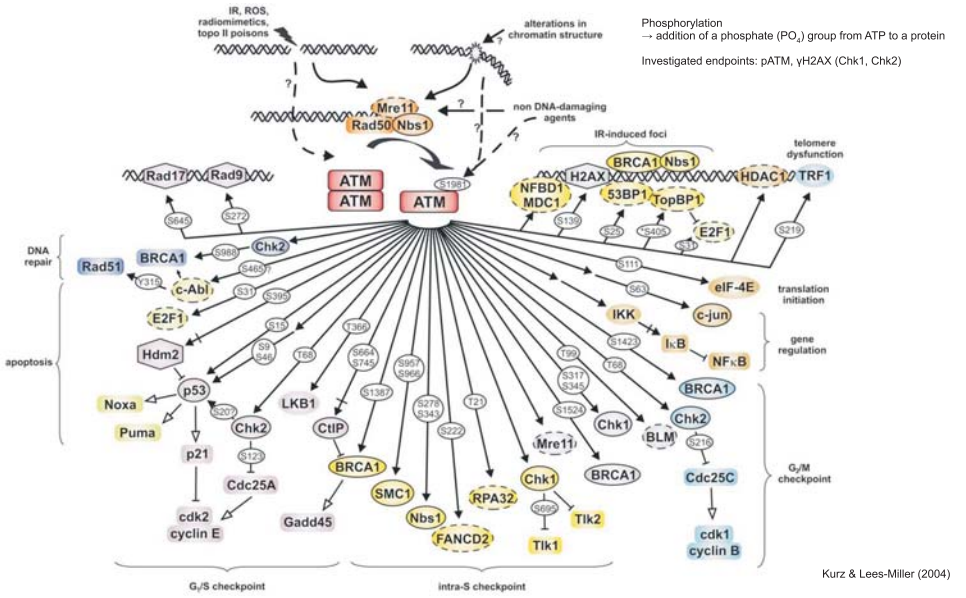
The cosmic radiation environment is significantly different from that found terrestrially. Cosmic rays primarily consist of high-energy charged particles, such as protons, alpha and heavier particles, originating from several sources, including GCR, SPE and trapped radiation belts. Some of these high-LET radiations inflict greater biological damage than that resulting from typical terrestrial radiation hazards. Future expeditions into interplanetary space will place crews at increased risk of exposure compared to the current short duration LEO missions. Our knowledge of biological effects of high-LET particles is still developing. Research in this area may thus lead to the development of biological dosimeters and be pivotal in the design of exposure regulations and the assessment of health risk to astronaut crews.



With the availability of high-energy particle accelerators and the development of microbeams, the radiobiological bystander effect became of eminent interest. At low levels of exposure the communication of bioactive substances from irradiated to unirradiated cells can amplify the damage and cause significant deviations from linearity in the dose response relations.

MANIFESTATION OF DNA DOUBLE STRAND BREAKS

The ataxia-telangiectasia mutated (ATM) protein plays a key role in regulating the cellular response to ionising radiation. Double strand breaks (DSB) are detected by the MRN protein complex (Mre11, Rad50 and Nbs1). Activation of ATM occurs through its autophosphorylation on serine 1981 (→ pATM) and conversion of ATM from an inactive dimer to an active, monomeric form. Once activated, ATM can phosphorylate targets at the DSB (e.g. histone H2AX → γH2AX) or, possibly, be released to phosphorylate downstream targets that modulate numerous damage response pathways, most notably cell cycle checkpoints (Chk1, Chk2) that lead to DNA damage-induced arrest at G_1/S, S, and G_2/M phases. pATM and γH2AX indicate the presence of DSBs.



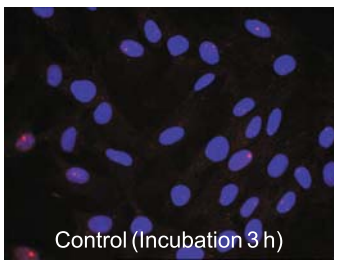
HIGH-ENERGY CHARGED PARTICLE EXPOSURES

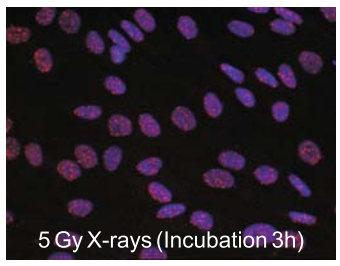
All exposures to high-energy charged particles were realised at the Heavy Ion Medical Accelerator (HIMAC) of the National Institute of Radiological Sciences (NIRS) in Chiba, Japan. The continuous support of Yukio Uchihori, Ryuichi Okayasu, Nakahiro Yasuda, Hisashi Kitamura, Maki Okada and the HIMAC operating staff is gratefully acknowledged. Variation of LET was achieved by means of attenuating PMMA absorbers.

^{4}He	150 MeV/n	2.2 keV/µm
^{12}C	290 MeV/n	13.1 keV/µm
^{28}Si	490 MeV/n	56.7 keV/µm ; 100.0 keV/µm
^{56}Fe	500 MeV/n	197.4 keV/µm ; 300.0 keV/µm

Irradiation → Incubation 2-3 h (cell detects damage & initiates DNA repair) → Fixation (stop of biochemical processes) → Cell staining (immunofluorescence or -chemistry) → Microscopy

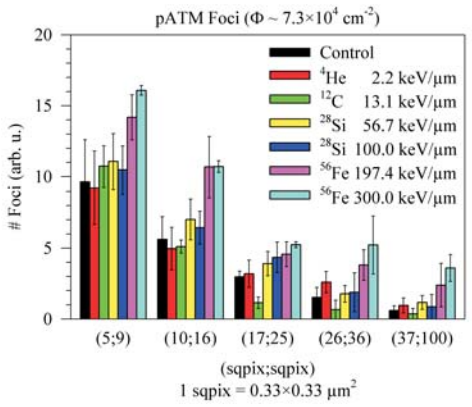
DSB VISUALIZATION: IMMUNOFLUORESCENCE

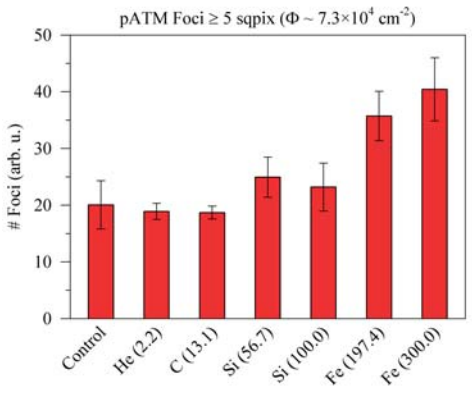




Rhodamine-stained pATM in normal human fibroblasts after 5 Gy X-rays, compared to a mock-irradiated control sample. pATM forms radiation-induced foci which co-localise with DSBs.

QUANTIFICATION OF pATM & γH2AX IMMUNOFLUORESCENCE (INCUBATION 2 h)





pATM foci are scored depending on their size. At least 600 fibroblasts were evaluated for each ion type and LET value. For all sizes, ^{56}Fe-irradiated cells show significantly more foci per nucleus than control as well as ^{4}He- and ^{12}C-irradiated samples.

The number of foci and cells are scored independently. Therefore, a direct correlation between damaged and cells with particle hits cannot be achieved.

Summing up all foci sizes, ^{56}Fe-irradiated samples yield about twice the number of foci than the control or ^{4}He-irradiated cells. These data indicate that the early cellular response to high-LET radiation is modulated by the energy deposition of the particle in the cell.

Percentage of fibroblasts with visible γH2AX foci is illustrated. About 600 cells were scored for each bar in the charts. No standard deviations are given due to the fact that the experiment has as yet only been performed once. Obviously, the number of damaged cells strongly depends on LET.

The right-hand diagram compares the percentage of cells with γH2AX foci above the level of the control sample (i.e. the cells affected by the radiation) with the cells with particle hits in their nucleus (calculated). The number of damaged cells after exposure to ^{56}Fe clearly exceeds the number of cells with direct hits, indicating a bystander response.