Fiscal Year:	FY 2008	**Task Last Updated:**	FY 08/08/2007
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Project Title:	Molecular and Cellular Effects of Heavy Ion Fragmentation due to Shielding		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Radiation Biology		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **SR**:Space Radiation		
Human Research Program Risks:	(1) **ARS**:Risk of Acute Radiation Syndromes Due to Solar Particle Events (SPEs) (2) **Cancer**:Risk of Radiation Carcinogenesis (3) **CNS**:Risk of Acute (In-flight) and Late Central Nervous System Effects from Radiation Exposure (4) **Degen**:Risk of Cardiovascular Disease and Other Degenerative Tissue Effects From Radiation Exposure and Secondary Spaceflight Stressors		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Project Type:	GROUND	**Solicitation / Funding Source:**	2004 Radiation Biology NNH04ZUU005N
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Contact Monitor:		**Contact Phone:**	
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Flight Assignment:	NOTE: Received NCE to 9/30/2010 per A. Chu/ARC (8/09) NOTE: Changed Division and Discipline/Program to HRP as of FY2006, per program changes at that time, per JSC/A. Chu-ARC (jvp 4/2009)		
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Task Description:	Galactic cosmic rays (GCRs) represent a major risk to human crews on long-term missions outside the Earth's magnetic field. The GCR consists of protons, helium nuclei and HZE (High Z and Energy) particles such as iron. Understanding the radiobiology HZE particles is of enormous interest as the energy of these particles can be sufficient in many cases to penetrate the spacecraft hull and interior materials. While traversing through matter, high energy radiation fragments into a large number of secondary particles with generally lower energy but with higher ranges and biological effects than the incident cosmic rays. Therefore, an exact knowledge of the biological effects of shielding is important not only for understanding the risks to humans on space flights but also for determining optimal shielding for space crafts. Previous studies have used relatively late end points such as chromosome aberrations and cell survival to elucidate the biological consequences of fragmentation due to shielding. The early response of a mammalian cell to ionizing radiation has recently been very clearly elucidated at the molecular level in the context of the relocation and modification of damage-responsive factors and these very early events have a very important bearing on the repair of DNA damage and the ultimate fate of the cell. In this proposal we aim to study the biological effects of shielding using these pertinent early molecular responses as end points. Specific Aims are: 1) To test the hypothesis that shielded heavy ions may result in more complex DNA damage to the cells as compared to unshielded heavy ions, 2) To test the hypothesis that the molecular response to shielded radiation is different from that induced by unshielded radiation, and 3) To test the hypothesis that shielded radiation may have more deleterious effects on the cell as compared to unshielded radiation and to elucidate the mechanisms involved in repair of DNA damage. Studies carried out in NSRL at Brookhaven National Laboratory during 2006 and 20007 (NSRL6A-7B) indicate that significant differences exist between DNA damage caused by unshielded Fe particles versus particles that have passed through different shielding materials. Our studies have also begun to elucidate the molecular and cellular consequences of the fragmentation of HZE particles during passage through shielding materials.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Galactic cosmic rays (GCRs) represent a major risk to human crews on long-term missions outside the Earth's magnetic field. The GCR consists of protons, helium nuclei and HZE (High Z and Energy) particles such as iron ions. Understanding the radiobiology of HZE particles is of enormous interest as the energy of these particles can be sufficient in many cases to penetrate the spacecraft hull and interior materials. While traversing through matter, HZE particles fragment into a large number of secondary particles with generally lower energy but with higher ranges and biological effects than the incident cosmic rays. Therefore, an exact knowledge of the biological effects of shielding is important not only for understanding the risks to humans on space flights but also for determining optimal shielding for space crafts. Previous studies have used relatively late end points such as chromosome aberrations and cells survival to elucidate the biological consequences of fragmentation due to shielding. The early response of a mammalian cell to ionizing radiation has recently been very clearly elucidated at the molecular level especially, the relocation and modification of damage-responsive factors at DNA-damage sites and these very early events have a very important bearing on the repair of DNA damage and the ultimate fate of the cell. In this proposal, we are studying the biological effects of shielding using these pertinent early molecular responses as end points. With these approaches, we can not only verify the immediate biological effects of beam fragmentation through shielding but can also estimate the efficacy of shielding materials.
	The HZE (High Z and Energy) component of Galactic cosmic rays (GCRs) represent a major risk to human crews on long-term missions outside the Earth's magnetic field. Understanding the radiobiology of HZE particles is of enormous interest as the energy of these particles can be sufficient in many cases to penetrate the spacecraft hull and interior materials. In this project, we are using pertinent DNA damage response (DDR) events not only to verify the immediate biological effects of beam fragmentation through shielding but also to estimate the efficacy of shielding materials. Summary of preliminary results obtained. Preliminary studies carried out during NSRL6A and 6B runs indicated that significant differences might exist between DNA damage caused by unshielded Fe particles versus particles that have passed through different shielding materials. These preliminary results were reconfirmed during the NSRL 6C, 7A and 7B runs. In addition, studies were initiated to elucidate the molecular and cellular consequences of DNA damage by the fragmentation products of Fe ions that have traversed shielding materials. General Experimental Strategy: Cells, Irradiation, Dosimetry and Shielding In this study, we used Fe (56) ions with a kinetic energy of approximately 1 GeV/nucleon at the NSRL (LET 150 keV/micrometers) to irradiate human skin fibroblasts (HSFs) after traversal through specific shielding materials. Two end points were used to visualize DSBs and to quantify its repair: 1) phosphorylation of histone H2AX and 2) recruitment of 53BP1. To understand the long-term consequences of irradiation of cells with fragmented Fe ions, endpoints such as activation of cell cycle checkpoints, senescence and cell death were quantified. For this study, we chose three different materials – 3 cm of aluminum (Al, Z=13), 3 cm of lead (Pb, Z=82), and 19 cm of polyethylene (CH2; Z of C=6; Z of H=1). 3 cm Al is representative of hull material on the Space Shuttle and the International Space Station (ISS) and does not substantially fragment or change the LET of HZE particles traversing it. 3 cm Pb, increases the LET of 1 GeV/nucleon Fe from 150 to almost 200 keV/micrometers with modest fragmentation. 19 cm of polyethylene (PE), a favored shielding material due to its high hydrogen content and a tissue surrogate for the same reason, was calculated to fragment most of the Fe beam; however, the surviving beam particles have approximately the same LET as beam particles after passing through 3 cm Pb. The materials were placed 3 cm upstream of the biological sample and surviving beam ions and charged fragmentation products were measured by solid state detectors at the same time as the biological experiments. Aim 1. To test the hypothesis that fragmented heavy ions may result in more complex DNA damage to the cells as compared to unfragmented heavy ions. 1. In order to establish a baseline with which to compare the effect of shielding on Fe ions, we visualized the extent of DNA damage induced by 1 GeV Fe and compared this with damage induced by gamma rays. Immunofluorescence staining of irradiated cells in combination with confocal microscopy and modeling of Z-stacks with Imaris software was utilized to generate 3D reconstructions of DNA damage areas.

Task Progress:	2. We irradiated cells through the different shielding materials and generated 3D reconstructions of the ensuing DNA damage. Cells irradiated through Al, i.e. by radiation only slightly modified compared to the beam, typically exhibit dense tracks of DNA damage indistinguishable from those produced by the beam alone. Irradiation behind Pb results in denser patterns of DNA damage, presumably due to the higher LET of the surviving beam particles. By contrast, the majority of cells irradiated behind PE do not exhibit discrete DNA-damage tracks, even though the LET of the surviving beam particles is almost the same as with Pb; diffuse areas of DNA damage, heterogeneous in their volume and distribution, are observed instead. 3. We find that cells irradiated with 1 Gy of gamma rays are mostly able to complete DSB repair by 12 h as has been demonstrated before. In contrast, cells irradiated with 1 Gy of Fe ions are unable to repair approximately 30 percent of the initial DNA damage incurred by 12 h; no evidence of further repair is seen after 12 h. We next tried to answer the question of whether fragmentation of a high LET ion into multiple particles, albeit of lower LETs, affects the ability of a cell to repair the ensuing DNA damage. We find that Al has little effect on DNA repair kinetics, which suggests that, by this criterion, the ISS hull affords little protection against HZE. Pb results in even slower kinetics of DNA repair, consistent with the observed increase in LET. The heterogeneous mix of DNA damage induced downstream of PE are repaired with somewhat faster kinetics; we assume that the faster repair is due to the generation of particles with lower LET due to fragmentation through PE; however, the DNA damage is not repaired to completion. Aim 2. To test the hypothesis that the molecular response to mixed field radiation due to nuclear fragmentation is different from that induced by homogeneous heavy ion radiation. 1. In pilot experiments, we have been able successfully study the localization and activation of DNA-PK at Fe particle induced damage with/without shielding. Studies involving ATM are planned for future runs. 2. We have been able to study the co-localization of 53BP1 with H2AX in pilot studies involving beam modification through the three different shielding materials. Detailed experiments on other modulators are being planned for the NSRL8 runs. 3. We examined the activation of p53 as a first step towards understanding the long-term consequences of the failure to repair Fe-induced DNA damage especially those caused by particle fragmentation. HSFs irradiated with gamma rays display rapid and transient p53 accumulation and phosphorylation at ser 15 and transient induction of the cyclin-dependent kinase (CDK) inhibitor p21 (Waf1/Cip1), a downstream target of p53. In contrast, cells irradiated with the unmodified Fe beam display a biphasic response with an initial transient response similar to that seen with gamma rays and a second sustained response starting at about 2 days post-irradiation and lasting for at least 10 days. A high level of the CDK inhibitor p16 (INK4a), that independently arrests cell proliferation in response to stress, is also induced during this stage. The p53 response pattern is largely unaltered after irradiation by the relatively unfragmented beams produced by Al or Pb. Interestingly, irradiation through PE shielding also results in a biphasic response; however, the second phase is modestly attenuated. Aim 3. To test the hypothesis that mixed field radiation may have more deleterious effects on the cell as compared to unfragmented radiation and to elucidate the mechanisms involved in repair of DNA damage. 1. We have initiated studies with cell lines deficient in specific DNA repair pathways to understand the contribution of these pathways to the repair of fragmented radaition. Specifically, we are utilizing knockout mouse cells with the following genotypes: wild type, DNA-PKcs-/-, and Atm-/-. As a first step, we have characterized the DNA repair response of these lines to proton irradiation to establish the inherent repair capabilities of the lines. As expected, repair of proton-induced DNA damage follows slower kinetics in DNA-PK-/- cells. In future runs we plan to use these lines to delineate the contribution of specific pathways in the repair of fragmented radiation. 2. Detailed colony survival assays were carried out with HSF cells irradiated with gamma rays, 1 GeV protons, 1 GeV Chlorine, and 1 GeV Fe (with no shielding or with Al, Pb or PE shielding) without any shielding or with Al, Pb, or PE shielding. HSFs are extremely sensitive to Fe ions as compared to gamma rays or protons while Cl ions result in intermediate radiation sensitivity which is in keeping with the LETs of these individual ions. As would be expected from our results, modifying the incident particle by passage through the various shields does not result in major differences in survival over unshielded ions. 3. We have characterized the induction of senescence in HSFs as a consequence of radiation using the Senescence-associated beta-galactosidase activity assay. We find that Fe-irradiated cells display a very high percentage of intensely-staining SA beta-Gal-positive cells at 10 d post-irradiation (~84%). This is clearly a consequence of the sustained p53 activation and p21/p16 induction observed in these cells. Induction of senescence is largely unaltered after irradiation by the relatively unfragmented beams produced by Al or Pb (data not shown). Consequently, irradiation by a highly fragmented beam (through PE) results in a modest decrease in the percentage of SA beta-Gal-positive cells at 10 day post-irradiation (~67%) thereby validating the importance, from a radiation protection standpoint, of shielding material that can fragment the incident heavy ion.

Bibliography Type:	Description: (Last Updated: 04/27/2023)
Abstracts for Journals and Proceedings	Mukherjee B, Miller J, Burma S. "The DNA damage response to HZE particles and its modulation by shielding." Presented at The 18th NASA Space Radiation Investigator's Workshop, Rohnert Park, CA, July 2007. NASA Space Radiation Investigator's Workshop , July, 2007. , Jul-2007
Abstracts for Journals and Proceedings	Mukherjee B, Miller J, Burma S. "The DNA damage response to HZE particles and its modulation by shielding." Presented at The International Conference of Radiation Research, San Francisco (July, 2007). The International Conference of Radiation Research, abstracts, 2007. , Jul-2007
Articles in Peer-reviewed Journals	Mukherjee B, Miller J, Burma S. "Modulation of the DNA damage response to HZE particles by shielding." Submitted to DNA Repair, May 2007. , May-2007