Fiscal Year:	FY 2017	**Task Last Updated:**	FY 01/05/2017
PI Name:	Cornforth, Michael Ph.D.		
Project Title:	Molecular Characterization of Transmissible Chromosome Aberrations Produced By Ions of Intermediate and High Atomic Number		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Radiation health		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **SR**:Space Radiation		
Human Research Program Risks:	(1) **Cancer**:Risk of Radiation Carcinogenesis		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	77555-5302	**Congressional District:**	14
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2013-14 HERO NNJ13ZSA002N-RADIATION
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No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA JSC
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Flight Program:			
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Key Personnel Changes/Previous PI:	January 2016: No changes.		
COI Name (Institution):	Loucas, Bradford Ph.D. (University of Texas Medical Branch, Galveston)		
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Task Description:	During deep space exploration, personnel will be exposed to charged particles of intermediate and high atomic number, often collectively referred to as densely ionizing radiations. For a given dose these are almost certainly more likely to cause cancer than the sparsely ionizing types of radiation typically encountered on Earth, such as x- and gamma rays. Since it is not possible to determine directly the carcinogenic potential of such radiations, it becomes necessary to rely on surrogate experimental systems to provide this information. For a number of reasons, the formation of nonlethal (transmissible) chromosome aberrations, mainly reciprocal translocations and inversions, is considered by many to represent the best surrogate endpoint. And yet, only recently have we begun to really understand the molecular processes governing their formation, including possible differences that probably exist in the way that aberrations produced by sparsely- versus densely-ionizing radiations are formed. We propose using advanced molecular methods, including genome sequencing, to characterize structural changes to the DNA of human cells that accompany the formation of transmissible chromosome aberrations caused by exposure to various types of radiation likely to be encountered in deep space.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Radiation-induced reciprocal chromosome translocations and inversions are particularly important in that regard as it relates to manned space activities. In addition to causing cancer, their appearance also accompanies ongoing genome instability processes associated with its progression. The fact that these particular chromosome aberrations are transmissible (non-lethal) also makes them ideal candidate biomarkers of accumulated radiation exposure. We argue that molecular analysis of breakpoint junctions formed as the result of translocations and inversions is vital to understanding the process of exchange aberration formation, since it is here where underlying repair/misrepair pathways leave their “molecular fingerprints.” Regarding relevance to NASA’s concerns, the study of chromosome aberrations stands to tell us much about mechanisms underlying the cancer process itself. The relationship between particle energy/track structure and radiogenic changes to the genome represents an important first step in understanding 1) basic dose-response relationships at low fluences and 2) fundamental carcinogenic processes that may ultimately form the basis for subsequent mitigation strategies.
Task Progress:	Objective 1 of this proposal involves the isolation and cytogenetic characterization of cell clones to be used in molecular analysis of chromosomal inversions and translocations. We have now collected and cryopreserved 20 human cell clones that represent the survival of single cells exposed to various ionizing radiations. These cell clones harbor a range of nonlethal chromosome aberrations, mostly translocations and inversions. In addition to previously isolated clones surviving exposure to gamma rays and 56Fe ions, we also now have data for clones whose progenitor cells were exposed to 0.2 Gy of 1.5 MeV 7Li ions delivered at NASA Space Radiation Laboratory (NSRL). These were sent to Colorado State University (CSU) for Directional Genomic Hybridization (dGH), a technique that allows us to interrogate the genome for chromosomal inversions. Two of the 7Li clones tested positive for one or more inversions, including clone K1-Li-04. Twelve clones were karyotyped by G-banding at the University of Virginia, which is necessary to localize the breakpoints of exchanges to within 5-10 megabase pairs of DNA. Working together with KromaTid Inc., we have now have access to a single-color dGH probe set covering chromosomes 1, 2, and 3, 7, and X, which should increase dramatically our ability to screen for radiation induced inversions. We anticipate the construction of 3-color/3-chromosome dGH probe sets in the near future. Objective 2 of this proposal involves the molecular characterization of these clones through the use of Next-Generation Sequencing (NGS), in order to determine the nature of the illegitimate junctions formed at the DNA level. We had previously hoped that we had identified the translocation breakpoint in clone K1-400 C4 by NGS. Unfortunately these efforts failed to produce results consistent with the known cytogenetic location of the translocation. The biggest problem we faced was the preponderance of false-positive SV calls to the reference genome. In other words, initial analysis left us with thousands of false-positive results. Since then, we incorporated several changes to our approach that finally produced successful results in one of the clones. This included a newer and improved version of the bioinformatic software used to compare DNA from the irradiated clones, with that of normal DNA. In order to correct for various reference mapping artifacts, we filtered out false positive SVs (structure variants) that occurred from reference mapping artifacts. We did this by comparing potential SVs found using this software to those in a panel of 5 normal DNA samples: that of a control clone from this study and 4 normal samples from the International Cancer Genome Consortium (ICGC). This reduced the total number of potential translocations to a few dozen. We further reduced the number of candidate translocations down to only one position that agreed with the cytogenetic position of the breakpoint at t(3q26.2;4q31.1) by requiring at least two supporting reads for each filtered sequence. PCR primers were made flanking this presumptive breakpoint, and amplified fragments were Sanger-sequenced by its chromatography profile. The one translocation that we did manage to sequence shared a short four-basepair stretch of DNA sequence homology at the breakpoint junction between chromosome 3 and 4. This suggests that nonhomologous end-joining was responsible for the formation of this translocation, although more clones will need to be sequenced before we reach a consensus mechanism for this type of radiation damage under study. We have determined that most all of the challenges we faced can be overcome by starting with a library with larger inserts. So we have now assembled a mate-pair library, which has been constructed and sequenced at the University of Texas Southwestern. It is currently being interrogated with various bioinformatic algorithms. We expect that these changes will dramatically increase the throughput of our experimental system, and allow for the sequencing of several other translocations and inversion in a timely manner.
Bibliography Type:	Description: (Last Updated: 06/11/2025)
Abstracts for Journals and Proceedings	Shuryak I, Loucas BD, Cornforth MN. "Seeking Beta: Experimental Considerations and Theoretical Implications Regarding the Detection of Curvature in Dose Response Relationships for True Simple Chromosome Interchanges." Poster presentation at the 62nd Annual Meeting of the Radiation Research Society, Big Island, Hawaii, October 16-19, 2016. 62nd Annual Meeting of the Radiation Research Society, Big Island, Hawaii, October 16-19, 2016. , Oct-2016

Articles in Peer-reviewed Journals	Loucas BD, Shuryak I, Cornforth MN. "Three-color chromosome painting as seen through the eyes of mFISH: Another look at radiation-induced exchanges and their conversion to whole-genome equivalency." Frontiers in Oncology. 2016 Mar 15;6:52. eCollection 2016. http://dx.doi.org/10.3389/fonc.2016.00052 ; PubMed PMID: 27014627; PubMed Central PMCID: PMC4791380 , Mar-2016
Articles in Peer-reviewed Journals	Shuryak I, Loucas BD, Cornforth MN. "Seeking beta: Experimental considerations and theoretical implications regarding the detection of curvature in dose response relationships for chromosome aberrations." Radiation Research. In press as of January 2017. , Jan-2017