Fiscal Year:	FY 2020	**Task Last Updated:**	FY 01/09/2020
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
Start Date:	03/11/2015	**End Date:**	01/10/2021
No. of Post Docs:	0	**No. of PhD Degrees:**	0
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
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:			
Flight Assignment:	NOTE: Extended to 1/10/2021 per NSSC information (Ed., 1/21/2020) NOTE: Extended to 3/10/2020 per NSSC information (Ed., 3/12/19)		
Key Personnel Changes/Previous PI:	January 2016: No changes.		
COI Name (Institution):	Loucas, Bradford Ph.D. (University of Texas Medical Branch, Galveston)		
Grant/Contract No.:	NNX15AG74G		
Performance Goal No.:			
Performance Goal Text:			

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 they relate to crewed space activities. In addition to causing cancer, their appearance also accompanies ongoing genome instability processes associated with their 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:	Following negotiations with NASA management, this project was reduced to two objectives. Objective 1 of this proposal involves the Isolation and cytogenetic characterization of cell clones to be used in further molecular analysis of chromosomal inversions and translocations. We have collected and cryopreserved several human cell clones that represent the survival and clonal expansion of single cells exposed to gamma rays, 56Fe and 7Li ions. These cell clones harbor a range of transmissible chromosome translocations and inversions. In light of more pressing challenges related to Objective 2, we consider the number of clones representing exposure to the various radiations used to be sufficient. Aside from some incidental inversion analysis by directional genomic hybridization (dGH), Objective 1 has been completed to the point where current efforts were focused on Objective 2. 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. Sanger sequencing at the base-pair level from the amplified fragment from a paired-end library in clone K1-400C4 showed a 4 bp microhomology at the t(3;4) translocation junction of clone K1-400C4, supporting the idea that such rearrangements are characteristic of microhomology-mediated nonhomologous endjoining (mmNHEJ) misrepair pathways. Significantly, the breakpoint on chromosome 3 mapped to an LTR sequence, while that on chromosome 4 mapped within a LINE element. To our knowledge, this is the very first report involving the sequencing and validation of a known radiation-induced translocation in human cells using modern massively parallel sequencing (Cornforth et al., Radiat Res, 2018. 190(1): p. 88-97). We still needed to establish mmNHEJ as a consensus mechanism for exchange aberration formation produced by gamma rays, and also whether similar mechanisms underly the fomation of such rearrangements following exposure to densely ionizing (high LET (linear energy transfer)) 56Fe and 7Li ions listed in the proposal. This analysis took the better part of three years to accomplish, which is far too slow to meet the objectives of the grant proposal. The significant difficulties we experienced in sequencing this rearrangement can now be understood by considering the fact that both breakpoint junctions of this reciprocal translocation occurred in repetitive DNA. For example, the chromosome 3 breakpoint mapped to an LTR sequence and the chromosome 4 sequence mapped within a LINE element. Repeat elements are notorious in causing difficulties for all next-generation sequencing approaches. In hindsight, it is apparent that this would be especially true for the short-read mate-pair approach we were using at the time, which led to thousands of false-positive calls to the reference genome. Moreover, we realized, given the large number of DNA repeats in the human genome, that radiation-induced breakpoints within repeats would be the rule, rather than the exception. Thus, we concluded in last year’s report that strategies making use of longer insert libraries would be necessary, such as sequencing based on mate-pair libraries, for which we provided proof-of-principle efficacy by re-sequencing clone K1-400C4, to show it yielded identical breakpoint assignments compared to paired-end methodologies. We also proposed investigating the use of single molecule/real-time sequencing (SMRT) in the context of its increased cost compared to paired-end and mate-pair strategies. In this report, we compared whole-genome sequencing via mate-pair sequencing as an alternative approach to identify SVs (structural variations) with a lower false positive rate. Mate-pair sequencing provides short read information for each end of DNA segments that are separated by ~2-4 kilobases in the genome and are therefore likely to be outside the repetitive region where the breakpoint(s) may be located. By comparing mate pair calls with those made from paired-end whole genome sequencing reads, we were able to identify the true-positive breakpoint and its position at single nucleotide resolution for the (3;4) reciprocal translocation of clone K1-400C4 with markedly fewer false-positive SV calls. We also identified the breakpoints of putative translocations in another 11 clones by mate pair sequencing alone, and many of those calls to the reference genome were supported by cytogenetic data (mFISH). It is important to note, however, that we have failed with either approach to call cytogenetically observable inversions as detected by cGH. More recently, we have successfully employed to SMRT (single molecule/real-time sequencing via PacBio) to characterize SVs. This newer alternative approach is capable of generating very long sequencing reads, which we think will help us to characterize inversions, and improve greatly the throughput of our workflow. This approach was, until recently, far too expensive to be applied for our studies, but with the Sequel System now in place at University of Texas Southwestern (UTSW), these costs have dropped by 80%, now making SMRT sequencing affordable for our project. We

	used SMRT to sequence the DNA of 6 clones and the parental nonirradiated control clone to comprehensively characterize the SVs. The DNA samples included one clone that was previously (and definitively) characterized by whole genome paired-end and mate pair sequencing (clone K1-400C4); the remaining 5 clones were previously analyzed by mate pair sequencing. Using a computational algorithm (pbsv) for long read sequencing data, we identified putative breakpoints in the genome of 6 clones. Importantly, we were able to accurately call all translocations that were cytogenetically observed. There were only a few additional/extraneous SVs called, which suggests that SMRT sequencing is plagued by few (if any) false-positive SVs calls. SMRT sequencing appears to be more specific than mate pair sequencing, which, in turn, has much higher specificity than our original short-read paired-end sequencing approach. We do note, however, that even SMRT sequencing failed to identify an inversion on chromosome 3 of clone K1-400C4. Since we have consistently failed to identify this SV with the three sequencing approaches utilized, we suspect there may a technical issue that prevents the identification of this inversion breakpoint. We speculate that this breakpoint may generate a secondary DNA sequence structure that prevents replication by DNA polymerases, which could prevent either the generation of fragments encompassing this breakpoint during sequencing library preparation or the incorporation of nucleotides during the sequencing reaction. Both of these scenarios would prevent the generation of sequencing reads that span the inversion breakpoint. In either case, we conclude that SMRT sequencing appears to be an efficient and specific approach, which is superior to short-read paired-end sequencing and mate pair sequencing for calling radiation-induced translocations definable with single nucleotide resolution. This will be the last report prior to the final close-out of this project, which we feel is on the cusp of an important breakthrough. It is expected that these newer two approaches outlined above, either by themselves, or likely in combination with short read technologies, will allow for a more rapid and accurate characterization of breakpoint junctions of radiation-induced large-scale structural variants to human chromosomes.
Bibliography Type:	Description: (Last Updated: 06/11/2025)
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