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Project Title:	Molecular Characterization of Transmissible Chromosome Aberrations Produced By Ions of Intermediate and High Atomic Number		
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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 they relate to manned 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:	There were originally three objectives in this proposal. Following subsequent negotiations with NASA management, two objectives remain. 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 nonlethal chromosome translocations and inversions. Incremental progress on Objective 1 was made. Specific Aim 1a of Objective 1 is largely complete. It is likely that no additional clones will need to be isolated, although we will not know for certain until Specific Aims 1b (G-banding and mFISH analysis) and 1c (inversion analysis) are complete. Progress focused on G-banding (Specific Aim 1b) of this Objective. In previous reports we showed banding analysis for clone K1-400C4. More recently, we partnered with Eli Williams, a clinical cytogeneticist at UVA (University of Virginia), who performed traditional G-banding on 12 additional clones. Data for four clones deriving from exposure to 56Fe ions is now included. G-banding is a vital step prior to sequencing, since it isolates breakpoint junctions to within 5-10 Mb pairs of DNA. This, in turn, is needed to screen out the plethora of false-positive calls that are typical of bioinformatic analysis of DNA sequencing data (Objective 2 below). The 7Li clones collected and cryopreserved still require further analysis by mFISH and G-banding, before they are ready for sequencing. Since KromaTid (the source of our dGH probes) now is capable of producing 3-color/3-chromosome dGH probe sets, we intended to use those for future inversion analysis, as soon as we fit our microscope with the appropriate filter sets. 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. After initial problems were encountered, last year we decided to concentrate our efforts on clone K1-400C4 in order to determine the best path forward. In the previous progress report we were able to tentatively identify one of the breakpoints in this rearrangement, but had not yet provided validation of its location. This is because a reciprocal translocation, such as the one identified in K1-400C4, actually contains two breakpoint junctions: in this case, both a t(3;4) and a t(4;3) component. Without information about both junctions, it was not possible to fully characterize the rearrangement at the DNA sequence level. In this report we describe below the successful characterization of both breakpoint elements, sequence across both of them, fully characterize the translocation, and suggest a more robust path forward for future studies. We increased the genomic depth of coverage from 8X to 30X and employed several new and more powerful bioinformatic software algorithms as they became available. We filtered out false positive SVs that occurred from reference mapping artifacts by comparing calls from clone K1-400C4 to those made in a panel of 5 normal DNA samples that included a control clone from this study, and 4 normal samples from the International Cancer Genome Consortium (ICGC). The consensus sequence from MPS for the best-supported candidate translocation indicated a 4bp AAGG overlap between the chromosome 3 and 4, in otherwise alignment between sequences at 3q26.2 and 4q31.1 in the Dec 2013 (GRCh38/hg38) human genome assembly. A BLAT alignment showed that the chromosome 3 sequence mapped to an LTR sequence (bases 70-133) and that the chromosome 4 sequence mapped within a LINE element (bases 1-73). These repetitive elements were responsible for large number of highly homologous matches within this region. [We believe this led to the plethora of false-positive calls that plagued our initial analysis.] We used the consensus sequence to design forward and reverse primers to enable PCR amplification across both possible junctions of the reciprocal exchange breakpoints. Sanger sequencing of the extracted and amplified PCR products revealed a translocation event that resulted in a 1 bp deletion of chromosome 4 and a 6 bp deletion of chromosome 3, with the translocation occurring within a 4 bp “AAGG” overlap between chromosome 3 and 4. We take our results as confirmation that t(3;4)(q26.3;q31) translocation has been successfully characterized at the DNA base pair level. These results are consistent with the rearrangement having been produced via microhomology-mediated nonhomologous endjoining (mmNHEJ). The finding that both breakpoints of the translocation in question occurred in repetitive DNA elements is also noteworthy. Although we did not anticipate this result, it is not altogether surprising, given that the

	majority of sequences in the mammalian genome are composed of DNA repeats of one type or another. Thus we can expect radiation-induced exchange breakpoints within DNA repeat sequences to be commonplace. We concluded that sequencing longer insert libraries may have a higher specificity for the identification of genomic regions containing repetitive DNA elements. We tested this strategy using Nextera Mate-Pair library preparation (Illumina) followed by Illumina sequencing at ~4X genomic overage. We generated and sequenced 13 mate-pair libraries from 12 additional translocation-bearing clones that derived following exposure to gamma rays, or to 1GeV/amu 56Fe ions. This analysis also included the K1-400C4 clone that was previously analyzed by whole genome paired-end sequencing as described above, and the parental K1 clone. By this new strategy, we were able to identify a (3;4) translocation breakpoint within K1-400C4, and it was identical to the one found earlier by paired-end analysis (Chr3:170,398,638; Chr4:139,706,161). Furthermore, when we compared the totality of breakpoint calls from the mate-pair sequencing with those of 30X whole genome sequencing for clone K1-400C4, only the (3;4) translocation breakpoint was shared between both sequencing data sets. Importantly also, from the mate-pair sequencing data for an additional 11 clones, we were also able to detect breakpoint regions that are supported by cytogenetic analysis. These results strongly suggest that the identification of shared SVs predicted from mate-pair and whole-genome sequencing reads provides a robust solution to eliminate false-positive SV calls and to identify true positive genomic rearrangements from sequencing data. This should lead to much higher throughput of SV analysis, compared to paired-end sequencing alone. To our knowledge, we are the first to actually characterize and validate a known radiation-induced translocation in human cells using modern massively parallel sequencing (MPS). Our working hypothesis is supported by the 4 bp microhomology at the t(3;4) translocation junction of clone K1-400C4, which is characteristic of mmNHEJ repair/misrepair pathways. A paper reporting these results has been submitted to a peer-reviewed journal.
Bibliography Type:	Description: (Last Updated: 06/11/2025)
Abstracts for Journals and Proceedings	Cornforth, MN, Kittler R, Loucas BD, Ray FA, Robinson E, Bedford JS, Williams ES, Spellman P, Anur P, Peto M, Wang N, Gray JW, Bailey SM. "Sequencing of a Transmissible Radiation-Induced Chromosome Translocation." Clastogenesis and carcinogenesis. Poster presented at the 63rd Annual Meeting of the Radiation Research Society, Grand Fiesta Americana Coral Beach, Cancun, Mexico, October 14-18, 2017. 63rd Annual Meeting of the Radiation Research Society, Grand Fiesta Americana Coral Beach, Cancun, Mexico, October 14-18, 2017. , Oct-2017
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Abstracts for Journals and Proceedings	Cornforth MN. "Molecular Characterization of Transmissible Chromosome Aberrations Produced by Ions of Intermediate and High Atomic Number." Talk during 2018 NASA Human Research Program Investigators' Workshop, Galveston, TX, January 22-25, 2018. 2018 NASA Human Research Program Investigators' Workshop, Galveston, TX, January 22-25, 2018. , Jan-2018
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