Fiscal Year:	FY 2009	**Task Last Updated:**	FY 08/05/2008
PI Name:	Wiese, Claudia Ph.D.		
Project Title:	A role for homologous recombination in complex DSB repair after HZE particles		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
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:	None		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
PI Email:	cwiese@lbl.gov ; Claudia.Wiese@colostate.edu	**Fax:**	FY 510-486-6816
PI Organization Type:	GOVERNMENT	**Phone:**	510-486-4024
Organization Name:	Lawrence Berkeley National Laboratory		
PI Address 1:	Mail Stop 977		
PI Address 2:	1 Cyclotron Road		
PI Web Page:			
City:	Berkeley	**State:**	CA
Zip Code:	94720-8099	**Congressional District:**	9
Comments:	For information purposes only--PI moved in June 2014; however, funding did not go with her. As of 6/1/14, Colorado State University, Department of Environmental and Radiological Health Sciences, 485 MRB - 1618 Campus Delivery, Fort Collins, CO 80523-1618, Office: (970) 491 7618, Email: Claudia.Wiese@colostate.edu		
Project Type:	GROUND	**Solicitation / Funding Source:**	2004 Radiation Biology NNH04ZUU005N
Start Date:	10/01/2005	**End Date:**	09/30/2010
No. of Post Docs:	0	**No. of PhD Degrees:**	
No. of PhD Candidates:	0	**No. of Master' Degrees:**	1
No. of Master's Candidates:	1	**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:	1	**Monitoring Center:**	NASA JSC
Contact Monitor:	Cucinott1a, Francis	**Contact Phone:**	281-483-0968
Contact Email:	noaccess@nasa.gov		
Flight Program:			
Flight Assignment:	NOTE: Received NCE to 9/30/2010, per J. Dardano/JSC (7/2009) NOTE: End date changed to 9/30/2009, per K. Willison (3/07) NOTE: Question re end date, per JSC; changed back to 9/30/2006 (1/07) NOTE: project extended for full length of proposal, per J. Dardano (2/06)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):			
Grant/Contract No.:	NNJ05HI36I		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Overall Rationale: The overall goal of this proposal is to investigate whether homologous recombinational DNA repair (HRR) contributes to the repair of double-strand breaks (DSBs) generated by the radiation types found in the space radiation environment. This proposal is a continuation of our previous work demonstrating that recombination is induced in human cells exposed to Fe ions. Here, we aim to directly assess the role of HRR in the repair of DNA damage after high linear energy transfer (LET) radiation in mammalian cells, an investigation that has never been carried out before. High LET charged particles deposit large amounts of energy along the ion trajectories, leading to the induction of highly localized DNA damage. These spatially correlated DSBs rejoin with slower kinetics and to less completeness than DSBs induced by low LET radiation. In mammalian cells, X-ray induced DSBs are primarily repaired by non-homologous end joining (NHEJ) in G1, but HRR plays a critical role in S- and G2-phases of the cell cycle. Several reports indirectly suggest that HRR, generally a precise form of DNA repair, plays an important role in the repair of correlated DSBs. Importantly, radiation-induced human tumors arise through a multi-step process of genetic change, and defects in HRR leading to the stimulation of error-prone DNA repair pathways may accelerate this process. For this reason, it is important to investigate directly whether alterations in the ability to perform HRR can sensitize humans to HZE particles. Approach: We will determine in syngeneic human cells whether defects in HRR affect the extent of cell killing and the mechanism of mutagenesis by densely ionizing Fe ions. RNA interference technology will be used to impair HRR (targeting XRCC3, Rad51D or Rad51) in the human lymphoid cell line WTK1. For comparison purposes, NHEJ will also be targeted, and X-rays will be used to test high vs. low LET radiation effects. The Fe particle-induced mutation frequencies will be determined at the autosomal TK1 locus and at the X-linked HPRT locus for parental WTK1 cells and for one representative derivative of WTK1 cells with a 'loss-of-function' phenotype for HRR and for NHEJ. Sets of TK1 mutants will be collected and the Fe ion-induced, X-ray-induced and spontaneous mutation spectra will be compared to discriminate between recombinational and deletional events. Furthermore, we will investigate in hamster and in human mutant cells whether impaired HRR enhances the cytotoxic effects of Fe ions. Wild-type CHO cells and CHO mutant cell lines impaired in HRR (Rad51D, XRCC3) or in NHEJ (DNA-PKcs) will be compared. Both asynchronous and synchronous cell cultures will be used and the relative contributions of both DNA repair pathways to cell survival will be assessed. DSB repair in CHO mutant cell lines and wild-type cells will be measured using a recently described assay that quantifies DSB rejoining by gH2AX foci formation. The effect of the loss of XRCC3 on the cellular sensitivity to Fe particles in an hTERT-immortalized human fibroblast strain will also be determined.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	
Task Progress:	The overall goal of this Research Project is to investigate whether homologous recombinational DNA repair (HR) contributes to the repair of double-strand breaks (DSBs) generated by the radiation types found in the space radiation environment. We hypothesize that, in some cases, correlated, clustered radiation damage, as induced by heavy charged (HZE) particles, requires the resection of the damaged DNA and the HR pathway, and therefore is not channeled into the non-homologous end-joining (NHEJ) pathway. Importantly, HR is a DNA repair pathway with close ties to cancer biology and crucial for maintaining genomic stability, limiting mutagenesis and preventing carcinogenesis. It is well established that conditions promoting reduced levels of HR compromise the fidelity of DSB repair and correlate with an elevated cancer risk. The risk of developing cancer is increased in individuals exposed to space radiation, and defects in HR, leading to the increased utilization of error-prone DNA repair pathways (i.e. NHEJ), are likely to contribute to this process. Therefore, it is a necessity to establish the relevance of HR to HZE radiation for better prediction of the astronaut's sensitivity to radiation carcinogenesis. In the last year we have conducted two experimental runs at BNL-NSRL using the 1 GeV/n Fe beam to test whether HR contributes to the repair of complex DNA DSBs induced by Fe ions. For comparison purposes, we have also conducted several experiments using X-rays. We have further refined our experimental approach in which we use RNAi transfection or conditional expression of shRNA to induce gene-specific knockdown of essential proteins functioning in HR. Furthermore, we now have included one NHEJ protein, XRCC4, a stimulator of DNA ligase IV, into our mutation analyses to better understand the complexity of our results under conditions when HR is compromised. We would like to point out that we have conducted our mutation experiments using comparatively low radiation doses (i.e. from 0.3 to 1.2 Gy) for both X-rays and Fe ions. In summary, our results using both human and hamster cell lines show that, after exposure to Fe ions, HR deficiency mildly but reproducibly decreases cellular survival and abrogates RAD51 foci formation. In addition, RAD51 foci formation in response to Fe ions is cell cycle regulated and occurs in S/G2 phase cells to facilitate strand exchange with the sister chromatid, but not in G1 cells. In rad51d-knockout hamster cells, the inability to recruit RAD51 into RAD51 foci after exposure to Fe ions and the increased cytotoxicity after Fe ion exposure are very likely to be intertwined, although this has not directly been investigated here. Human fibroblasts and epithelial cells depleted for RAD51D are impaired in RAD51 foci formation both after X-rays and after Fe ions. Our data on Fe ion-induced mutagenesis in HR-impaired human cells are complex and show that functional HR is a prerequisite for ensuring genome maintenance and proper repair of DNA damage induced by 1 GeV/n Fe ions in "permissive" human lymphoblastoid cells (i.e. p53 mutant WTK1 cells). Furthermore, our results clearly demonstrate that the susceptibility to mutagenesis is dependent on both the locus investigated (i.e. HPRT vs. TK) and the genetic background (i.e. wild type vs. defective HR). Compared to control transfected cells, we recover more TK mutants from HR defective WTK1 cells after exposure to Fe ions, suggesting that at this locus DNA repair fidelity is reduced significantly when decreased levels of HR proteins are expressed. We conclude that ability to properly perform HR after exposure to Fe ions is essential for limiting mutagenesis at an autosomal locus. Interestingly, simultaneous depletion of the NHEJ protein XRCC4 reduces TK mutation levels significantly when HR is impaired. Our results show that ablation of HR leads to an increase in NHEJ-driven mutagenesis at TK. Furthermore, in WTK1 cells XRCC4-dependent NHEJ also contributes to Fe ion-induced mutagenesis at the hemizygous HPRT locus, but defects in HR do not affect HPRT mutation levels after Fe ions at this hemizyous locus, indicating either that the contribution of HR-mediated mechanisms to mutagenesis at HPRT is minor, or that, under conditions of impaired HR, HPRT mutants derived from NHEJ events cannot be recovered. In wild type p53 TK6 cells, NHEJ contributes to mutagenesis at TK both after Fe ions and after X-rays, but alterations in the ability to perform HR apparently have no effect, suggesting that HR does not contribute to mutagenesis at this heterozygous locus (as expected) and may only play a minor role in repairing radiation-induced DSBs within TK or within the genomic region flanking TK. We consider the possibility that p53 wild type cells

	impaired in HR by depletion of RAD51D are unable to process endogenous replication damage and exogenous DNA damage due to Fe ion or X-ray exposure, and may escape the mutation analysis due to increased cell death. Taken together, ablation of HR greatly affects Fe ion-induced TK mutagenesis in p53 mutant but not in p53 wild type human lymphoblastoid cell lines. Since the inactivating p53 mutation in WTK1 cells is in the sequence-specific DNA binding domain and since the same mutation is observed in some human tumors, the phenotype of WTK1 cells is somewhat typical of many p53 mutant cells. It has been estimated that a certain fraction of cells within a healthy individual's body spontaneously acquire p53 mutations that then allow oncogenic transformation to occur. For this, mutation analyses in genetically predisposed cell lines can provide a useful means to derive radiation risk estimates for carcinogenesis in humans traveling to space, since these humans presumably already contain precancerous cells with p53 mutations.
Bibliography Type:	Description: (Last Updated: 04/11/2018)
Abstracts for Journals and Proceedings	Zafar F, Seidler SB, Kronenberg A, Schild D, Wiese C. "A role for homologous recombination in complex double-strand break repair after HZE particles." NASA Human Research Program Investigators' Workshop, League City, TX, February 4 - 6, 2008. Abstracts, NASA Human Research Program Investigators' Workshop, League City, TX, February 4 - 6, 2008. , Feb-2008
Abstracts for Journals and Proceedings	Zafar F, Seidler SB, Kronenberg A, Schild D, Wiese C. "Homologous recombination contributes to the repair of double-strand breaks induced by Fe-ions." 19th Annual NASA Space Radiation Investigators' Meeting, Philadelphia, PA, June 30 - July 2, 2008. Abstracts, 19th Annual NASA Space Radiation Investigators' Meeting, Philadelphia, PA, June 30 - July 2, 2008. , Jun-2008
Articles in Peer-reviewed Journals	Wiese C, Dray E, Groesser T, San Filippo J, Shi I, Collins DW, Tsai MS, Williams GJ, Rydberg B, Sung P, Schild D. "Promotion of homologous recombination and genomic stability by RAD51AP1 via RAD51 recombinase enhancement." Mol Cell. 2007 Nov 9;28(3):482-90. PMID: 17996711 , Nov-2007