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Low Dose Radiation Research Program

Workshop I

Low Dose Radiation Research Program
U.S. Department of Energy
Office of Biological and Environmental Research

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DOE Low Dose Radiation Research Program Workshop I

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Introduction to Workshop I

Welcome to the first DOE Low Dose Radiation Research Program workshop. This meeting is intended to be scientifically interesting, intellectually challenging, and an educational opportunity for all of us—benefits that will not come without your full and active participation in all presentations, discussions, and poster sessions. The workshop also is meant to be different from others you may have attended, but then the Low Dose Radiation Research Program is a unique program. It offers all of us the opportunity and challenge to make contributions that will impact the future of radiation risk policy in this country—not an easy or insignificant task. We have invited a wide range of individuals, from research scientists to citizen representatives to government bureaucrats to communication experts, to provide the kind of broad perspectives and interactions necessary for the program's success.

We believe that if all we do in this program is excellent science, we will fail. In the end, success will be measured by the impact of the program's science on radiation risk policy. The undertaking is still a scientific program, not a policy program. It will not generate new radiation risk policy or support policy research. Nevertheless, the science cannot be conducted in a vacuum. The project requires an awareness of what policymakers need to do their jobs and an understanding of public concerns and interests about radiation. It must be carried out, of course, under the scrutiny of scientific peer review.

This gathering will initiate dialogues that we hope will continue and grow in the coming years. The dialogue process, although not familiar to some scientists, will help to shape and direct the program. To assist us in maintaining and identifying some of these essential interactions, we are establishing a Low Dose Radiation Research Program Advisory Committee, a subcommittee of our office's Biology and Environmental Research Advisory Committee. This advisory group also will reflect the program's broad constituency from scientists to the public to risk policymakers and communicators.

Over the two days of the meeting, we look forward to discussing many interesting and stimulating topics, forging new collaborations and research partnerships, developing a broader understanding of public concerns and interests about radiation, and narrowing the gap between basic research and risk policy needs. Thank you for your interest in the Low Dose Radiation Research Program and for your participation in this important workshop.

Work hard. Share your views, your expertise, and your experience. Challenge your own ideas and your neighbor's ideas. Learn. Help us make this a better, stronger, and more credible research program. Enjoy.

David G. Thomassen, Ph.D.
Program Coordinator

Agenda

Time	Speaker	Program
Wednesday November 10		
1:00 - 1:20	David Thomassen, DOE Office of Biological and Environmental Research	Low Dose Radiation Research Program
1:20 - 2:10	Martha Krebs, Director, Office of Science and Mark Gilbertson, Office of Environmental Management	Welcome and Perspective
Beyond the Research Laboratory - Policy, Public, Press		
2:10 - 2:55	L. Manning Muntzing	Links Between Radiation Research and Risk Policy
2:55-3:15		BREAK
3:15-4:15	Mary Harlow and Samantha Dixion, Rocky Flats Radionuclide Soil Action Level Oversight Panel Jim Chestnut, Paducah, Kentucky	Public Perspectives on Radiation Risk
4:15-5:15	Charles R. Chappell, Vanderbilt University	Communicating Science - Scientists, the Media, the Public
5:30-7:30		Posters / hors d'oeuvres
Thursday November 11		
8:30 - 9:15	Antone Brooks, Washington State University	Low Dose Radiation Biology Research
9:15 - 9:30	David Thomassen	Charge to Breakout Groups
9:30 - 12:00		Breakout groups
12:00 - 1:30		Working Lunch
1:30 - 2:15	Peter Groer, University of Tennessee	Dose-Response Models
2:15 - 4:15		Breakout groups

Time	Speaker	Program
4:15 - 4:30	Rick Jostes	BEIR VII Update
4:30 - 5:00	Breakout group chairs	Breakout Group Update
5:00 - 7:00		Posters / hors d'oeuvres
Friday November 12		
8:30 - 9:15	William Farland, U.S. Environmental Protection Agency	Using Mechanistic Data to Develop Risk Policy
9:15 - 11:45		Breakout groups and reports
11:45 - 12:00	David Thomassen	Meeting Wrap-Up / Future Directions
12:00 - 2:00	Session chairs / note takers	Lunch / Prepare Breakout Group Summaries
	Advisory Committee meeting	Lunch / Program Review Wrap-Up

Poster Sessions

Poster Session 1
Wednesday, November 10
5:30 – 7:30 p.m.

Poster No.		
1	Barcellos-Hoff	Mechanisms of Tissue Response to Low Dose Ionizing Radiation Exposures: Bioinformatic Tools for Multiparametric Image Analysis
3	Bogen	DNA Damage vs. Cell Killing by Low-Dose-Rate Gamma Radiation Ultrasensitive Measures, and Implications for Mechanistically Modeled Cancer Risk
5	Braby	The Role of the Number and Spacing of Electron Tracks on the Consequences of Low Dose Irradiation
7	Brenner	The Risk of Cancer Induction Due to Routine Mammographic Screening
9	Brooks	Optimizing the Scientific, Regulatory and Societal Impact of the DOE Low Dose Radiation Research Program
11	Chatterjee	Biological Effects of Low-Dose and Low-Dose-Rate Radiation Exposures: An Integrated Theoretical and Experimental Approach
13	Flynn	Understanding the Issues in Communicating about Low Dose Radiation Exposure
15	Geard	Site Specific Microbeam Irradiation: Defining a Bystander Effect
17	Haber	Gene Targeting and Nonhomologous End-Joining in *Saccharomyces*
19	Hoel	Models of Radiation Induced Translocations Applied to Human Leukemia Risk Estimation
21	Michael	Low-Dose Studies with Focused X-rays in Cell and Tissue Models: Mechanisms of Bystander and Genomic Instability Responses

Poster No.		
23	Moolgavkar	Stochastic Models for Low-LET Radiation Risk Estimation at Low Dose and Dose-Rate
25	Orlando	A Novel, Spatially Resolved Cell Irradiator to Study Bystander and Adaptive Responses to Low-LET Radiation
27	Sachs	Cytogenetic Tests of Radiobiological Models Relating Epidemiologically Measurable Risks to Low-Dose Risks
29	Scott	Advanced Computational Approaches for Characterizing Stochastic Cellular Responses to Low-Dose, Low-Dose-Rate Exposures
31	Smith	Direct Characterization of the Differences between DNA Damage from Ionizing Radiation and Endogenous Oxidative Processes
33	Sutherland	DNA Damage Clusters in Low Level Radiation Responses of Human Cells
35	Wassom	Assessing Research on the Biological Effects of Low Dose Radiation Exposure to Evaluate Human Health Risks
37	Wilson	Track Structure Simulations for Selected-Cell Irradiation Studies

Poster Session 2
Thursday, November 11
5:00 – 7:00 p.m.

2	Bloom	The Dynamic Behavior of Broken Chromosomes in Yeast
4	Boothman	Nuclear apoJ/XIP8: A Cell Death Signal Regulated By p53 Induction Responses
6	Bull	Sensitivity to Radiation-Induced Cancer in Hemachromatosis
8	Chen	Genome Wide Gene Expression of LLIR and Biological Consequences
10	Cooper	Mechanisms of DNA Damage Responses to Low Dose Ionizing Radiation: Molecular, Biochemical, and Cellular Studies
12	Cornforth	Current Cytogenetic Issues Pertaining to Low Dose and Low Dose Rate
14	Dynan	Markers of the Low Dose Radiation Response

Poster No.		
16	Evans	Genomic Instability Induced by Low Dose Irradiation
18	Fornace	Development of a Functional Genomics Approach Employing Radiation-Induced Changes in Gene Expression to Monitor Cells after Low Dose and Low Dose-Rate Exposures
20	Goodwin	Biological Effects of Low Level Ionizing Radiation and Normal Oxidative Damage: The Same or Different?
22	Joiner	Mechanisms of Enhanced Cell Killing at Low Doses: Implications for Radiation Risk
24	Lehnert	Low Dose, Low Dose Rate Effects of Ionizing Radiation in Irradiated and Unirradiated Cells
26	Little	Effect of Low-Dose Alpha Irradiation in Human Cells: The Role of Induced Genes and the Bystander Effect
28	Mohrenweiser	DNA Repair Gene Variants: Understanding Mechanisms of Cellular Response and Estimating Individual Health Risk from Low-Dose Radiation Exposure
30	Morgan	Genetic Factors Affecting Susceptibility to Low-Dose Radiation
32	Redpath	Adaptive Response Against Spontaneous Neoplastic Transformation In Vitro Induced by Ionizing Radiation
34	Saffer	Linking Molecular Events to Cellular Responses at Low Dose Exposures
36	Schwartz	Radiation Sensitivity and Cancer Susceptibility
38	Thompson	Assessing Biological Function of DNA-Damage Response Genes
39	Tucker	Role of the “Adaptive Response” in Determining Health Risks from in Vivo Exposures to Low Doses of Ionizing Radiation
40	Wallace	Free Radical DNA Damage Produced Endogenously and by Low Dose Radiation in Human Cells: Quantitation, Consequences and Repair
41	Wyrobek	Molecular Mechanisms and Cellular Consequences of Low-Dose Exposure to DNA Damaging Agents

Breakout Group Assignments

Group 1

Mary Helen Barcellos-Hoff - Co-CHAIR
Les Braby
David Brenner
Dick Bull - SCRIBE
Aloke Chatterjee
Peg Coleman
Evan Douple
Bill Farland
Ludwig Feinendegen
Marv Frazier
Mark Gilbertson
Ray Johnson
Dave Kaufman
Pat Prasanna
Lowell Ralston
Les Redpath
Gordon Rogers
Bjorn Ryderg
Richard Moore
Kmiaki Sato
Jeff Schwartz
Paul Slovic
Jim Tucker
Susan Wallace – Co-CHAIR
Carol Worth
Walter Wilson

Group 2

Justine Alchowiak
Edouard Azzam
David Boothman – Co-CHAIR
Mike Cornforth
Samantha Dixion
Dan Drell
William Dynan
Helen Evans
Jim Flynn
Duane Fitzgerald
Eli Kraus
Nort Haberman
Judith Johnsrud
Rick Jostes
Shohei Kato
Barry Michael
Mike Boyd – Co-CHAIR
Peggy Olive
Dick Pelroy
Bobby Scott
Marvin Stodolsky
Philippe Voisin
James Walker
Tom Weber - SCRIBE
Andy Wyrobek

Group 3

Bruce Aronow
Ken Bogen
Tony Brooks - SCRIBE
Roger Cox
Albert Fornace
Arnold Gritzke
Peter Groer
Mary Harlow – Co-CHAIR
Cindy Jones
Arthur Katz
Peter Kirchner
Thomas Koval
Sree Kumar
Harvey Mohrenweiser
Bill Morgan – Co-CHAIR
V.Gio Nguyen
Tom Orlando
Jerry Puskin
Tom Radivoyevitch
Walter Schimmerling
Douglas Thrower
Dick Smith
Betsy Sutherland
Amy Wolfe

Group 4

Martin Adelson
Sally Admundson
David Chen
Jim Chestnut
Sharon Friedman
Michael Fry
Charles Geard – Co-CHAIR
Ed Goodwin
Kathy Held – Co-CHAIR
Vince Holahan
Michael Joiner
Bruce Lehnert
Margaret Lyday
Bob McGaughy
Suresh Moolgavkar
Larry Robinson
Susan Rose
Rainer Sachs
Jim Selkirk
Dan Stram
Larry Thompson
Mike Viola
Bruce Wachholz
Andy Wallo
John Wassom – SCRIBE

Charge and Questions for Breakout Groups

Charge

Use the diversity of expertise, opinions, and experience in each breakout group to identify ways for the DOE Low Dose Radiation Research Program to achieve its goal of supporting high quality, publicly acceptable research that underpins future policies to protect people from adverse health risks due to radiation exposure.

Questions and Challenges

Understanding the Research Portfolio and Program Goals

Each investigator funded by the Low Dose Radiation Research Program should present a brief, 'plain talk' description of their research project to their breakout group. The group is responsible for developing a general understanding of each project and for suggesting how it will contribute to future estimates of human health risk from radiation. The following first line of a future *New York Times* article describing the most successful outcome of the research should be completed for each project.

"In a major breakthrough, scientists today announced that ..."

Decisions about what research to fund in the Low Dose Radiation Research Program are currently made by (1) identifying the highest quality research using peer review and (2) selecting those projects that result in a balanced research portfolio and address both the short-term and long-term needs of the program. What are the advantages or disadvantages of

- Funding only the research that directly supports the development of radiation risk policy?
- Funding the best basic science and simply letting the results 'fall where they will?'

Communication - The Key to a Successful Research Investment

Everyone from J.Q. Public to the research scientist to the policy maker has concerns and expectations regarding risks from radiation and what we are doing to address those concerns and expectations. Knowing what these diverse groups want and need to know is an essential first step to successful communication that will, in some cases, require research.

- What does the public want to know and need to know?
- What do scientists want to know and need to know?
- What do policy makers want to know and need to know?

This research program and its long-term goal of contributing to the protection of human health will fail if the research is done in a vacuum. What things need to be done so that the public, scientists, and policy makers work together, both within their own groups and across groups, to ensure that radiation risk policy is based on the best possible science? How can we facilitate interaction and collaboration among these groups?

Building a Better, Stronger Research Program

Radiation, DOE, and regulatory policy all evoke strong, emotional reactions from many people. Even good science, while a rallying cry for scientists and, possibly, policy makers, may make some people suspicious that the scientists are simply trying to put something over on the American people. Given the public's current perceptions of DOE, radiation, regulatory policy, and even science, what things need to be done to ensure that the Low Dose Radiation Research Program supports science that

- Is believed and understood?
- Impacts regulatory choices?

What is missing or still needed in the Low Dose Radiation Research Program:

- Specific/new research tools?
- Additional research topics?
- Accessible information on radiation, research results, or the development of risk policy?
- A public forum for discussing radiation and health risk issues?
- Mechanisms to facilitate ongoing communication between the public, scientists, and policy makers?
- Educational tools? resources? curricula?
- Resources for journalists?

Response to Radiation Damage

1. Nuclear apoJ/XIP8: A Cell Death Signal Regulated By p53 Induction Responses

David A. Boothman and Bruce Aronow
Departments of Radiation Oncology and Pharmacology, Case Western Reserve University, BRB-326 East, 10900 Euclid Avenue, Cleveland, OH 44106-4942
dab30@po.cwru.edu

Summary: A low dose inducible protein has been found which induces cell death of genetically unstable cells. The protein is a sensitive indicator of low level radiation and its loss may indicate a prelude to the formation of cancer cells.

Abstract: We isolated apolipoprotein J [apoJ, also known as TRPM-2, SGP-2, and X-ray-inducible protein-8 (XIP8)], a protein implicated in apoptosis, tissue injury and aging, by Ku70 yeast two-hybrid cloning. Its interaction with Ku70 in vivo was confirmed by coimmuno-precipitation where Ku70 and Ku80 were associated with an ionizing radiation-inducible form of apoJ, nuclear apoJ/XIP8. Nuclear apoJ/XIP8 accumulated and colocalized with Ku70/Ku80 in the nuclei of irradiated MCF-7 cells by confocal microscopy. Cells over-expressing nuclear apoJ/XIP8 or its minimum Ku70 binding domain show reduced cell growth and significant lethality compared to GFP-alone constructs or apoJ/XIP8-GFP full-length protein expression wherein GFP fusion partially disrupted its interaction with Ku70. We recently demonstrated that induction of this protein can occur by as little as 2 cGy (rads). Furthermore, evidence will be presented that this protein is p53-regulated, since agents which induce p53 stimulate expression of this protein, E6 expression blocks its induction, and overexpression of wild-type, but not p73, p51, or mutant p53 forms, cause dramatic elevations in apoJ/XIP8 expression. Taken together, our results suggest that a p53-dependent formation of apoJ/XIP8-Ku70/Ku80 trimeric protein complexes represent a signal for cell death after low level IR stress.

2. The Risk of Cancer Induction Due to Routine Mammographic Screening

David J. Brenner, Steve Marino, and Charles Geard
Center for Radiological Research, Columbia University, 630 West 168th Street, New York, NY 10032
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Summary: To obtain realistic and credible risk estimates for breast-cancer mortality due to clinical mammographic imaging examinations.

Abstract: The aim of this work is to obtain realistic and credible risk estimates for breast-cancer mortality due to clinical mammographic imaging examinations. Given the increasing emphasis on clinical mammographic screening for breast cancer, it is of societal importance to provide realistic risk estimates — with realistic confidence bounds — for breast cancer

induction from routine mammographic X rays. Concern about this risk is a major factor (both from the perspective of the patient and the family physician) in the disturbingly large numbers of women who chose not to undergo routine mammographic screening.

Direct studies of mammographic screening programs have insufficient power to quantitate risks of mammographic screening, so estimates of the risks must be based on extrapolations from other cohorts (A-bomb survivors, medical irradiations). These other cohorts, however, were exposed to far higher-energy X or γ rays than the very low-energy X rays used in mammograms; *dose-for-dose, low-energy X rays are considerably more radiobiologically damaging than high-energy X or γ rays.* So it is likely that this risk estimation process under-estimates risks of screening mammograms — preliminary estimates are by a factor of 1.5-2.

We are using mechanistically-based techniques to extrapolate from those situations where radiation-induced breast-cancer risk estimates are better known (A-bomb survivors, medical irradiations), to risks from screening mammograms.

1. Experimental microdosimetric measurements are being used to characterize the energy-deposition characteristics of low-energy (5-28 keV) monoenergetic x rays. These studies are taking place at the X23A2 beam line of the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory. Based on these data, the biological effectiveness of realistic mammographic beams can be estimated relative to those for which breast-cancer risks have been estimated (e.g. A-bomb survivors, TB patients).
2. Direct radiobiological experiments are also underway at the NSLS of the RBE of these low-energy x rays, for the endpoints of chromosomal aberration formation and *in-vitro* oncogenic transformation, in mammalian cells.

The "bottom line" of these studies will be realistic estimates of the risk from routine screening mammograms, together with credible confidence limits that may reasonably be associated with these risk estimates.

3. Site Specific Microbeam Irradiation: Defining a Bystander Effect

Charles R. Geard, A.S. Ballajee, T.K. Hei, B. Ponnaiya, and S. Marino
Center for Radiological Research, Columbia University, New York, NY, USA
crg4@columbia.edu

Summary: A recently developed means of delivering charged particles to precise sites in or near cells [a microbeam], will be used to define the relative contributions of specific sub-cellular and/or extra-cellular sites to the damaging effects of known numbers of particles, including 1, the lowest possible radiation dose.

Abstract: The genetic, carcinogenic and cytotoxic effects of ionizing radiations have been assumed for many years to have their source in the direct induction of damage to DNA in the nuclei of cells. This basic paradigm has come into question in recent years as a consequence of studies with very low doses of alpha particles and of other studies with irradiated media. These findings of effects on presumably non-hit and hence not directly damaged cells has been termed a bystander effect. In no instance however could it be determined with any degree of certainty which cells were actually hit with a particle or where. We have developed a charged particle microbeam which provides a level of precision in terms of numbers of particles, sites of placement of particles, and temporal delivery

of particles which is unattainable with conventional radiation sources. That is, we have the means to address questions about the bystander effect in a definitive manner. Human fibroblasts have been irradiated with known numbers of particles through cell membranes, cytoplasm, nuclei and medium [trans-nuclear]; membranes, cytoplasm and medium [trans-cytoplasmic] and adjacent to cells [trans-medium]. In this way the relative contributions of each component in the cellular milieu to irradiation will be defined. Experiments to date have provided definitive proof of the reality of a radiation bystander effect but relative contributions of the components of the response pathway remain to be determined. Hence a basic paradigm in defining radiation responsiveness which is the basis for low dose extrapolations in the establishment of protection standards is under challenge.

4. Mechanisms of Enhanced Cell Killing at Low Doses: Implications for Radiation Risk

Michael C. Joiner, Peter J. Johnston, Brian Marples, Simon D. Scott, and George D. Wilson
Gray Laboratory Cancer Research Trust,
P.O. Box 100, Mount Vernon Hospital,
Northwood, Middlesex HA6 2JR, United Kingdom
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Summary: To understand why individual cells show very high sensitivity to the lethal effects of low radiation doses, and whether this protects the whole cell population by destroying mutated cells before they can propagate and cause cancer.

Abstract: We have determined previously that radiation sensitivity can be dose-dependent so that small acute exposures (and possibly exposures at very low dose rates) are more lethal per unit dose than larger exposures above a threshold (typically 5-40 cGy) where radioresistance increases. We have termed these dual phenomena low-dose hypersensitivity (HRS) and increased radioresistance (IRR) as the dose increases. HRS/IRR had been recorded in cell-survival studies with yeast, bacteria, protozoa, algae, higher plant cells and insect cells. However, we were first to demonstrate this phenomenon in mammalian cells and over the past decade, we have accumulated data indicating that HRS/IRR is widespread in both immortalized and non-immortalized human cells in vitro, and in animal normal-tissue models in vivo. More recently, research has revealed this phenomenon in the epidermis of patients undergoing radiotherapy for prostate cancer. We therefore believe that HRS may be the constitutive response of all normal cell systems to low-dose radiation exposures, which contrasts with the belief held for many years.

Our overall aim in this project is to gather understanding of the mechanisms underlying HRS/IRR. Little is currently known. However, there is now some direct evidence that this dose-dependent radiosensitivity phenomenon reflects changes in the amount, rate or type of DNA repair, rather than indirect mechanisms such as modulation of cell-cycle progression, growth characteristics or apoptosis. There is also indirect evidence that cell survival-related HRS/IRR in response to single doses might be a manifestation of the same underlying mechanism that determines the well-known adaptive response in the two-dose case, thus HRS can be removed by prior irradiation with both high- and low-LET radiations as well as a variety of other

stress-inducing agents such as hydrogen peroxide and chemotherapeutic agents. Changed expression of some genes, only in response to low and not high doses, may occur within a few hours of irradiation and this might be rapid enough to explain this.

Our goals in this project are therefore:

1. Identify which aspects of DNA repair (amount, rate and type) determine HRS/IRR,
2. Investigate the known link we have discovered between the extent of HRS/IRR and position in the cell cycle, focusing on changes in DNA structure and conformation which may modulate DNA repair,
3. Use the results from studies in (1) and (2) to distinguish, if necessary, between HRS/IRR and the adaptive response. The aim is to finally determine if these are separate or interlinked phenomena.
4. Use the results from studies in (1), (2) and (3) to propose a mechanism to explain HRS/IRR.

Net cancer risk is a balance between cell transformation and cell kill. Our hypersensitive low-dose cell-survival responses suggest that cell lethality could more than compensate for transformation at low-LET radiation doses up to about 10 cGy. This would lead to a non-linear, threshold, dose-risk relationship implying that the cancer risk from small radiation doses (<10 cGy) could be overestimated in specific cases. This out (<10 cGy) could be overestimated in specific cases. This outcome would have major cost-reduction implications for the EM program.

5. Effect of Low-Dose Alpha Irradiation in Human Cells: The Role of Induced Genes and the Bystander Effect

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Summary: The objective of this research program is to examine changes in gene expression and the induction of genetic damage in populations of normal human cells exposed to very low doses of alpha-particles whereby only a small fraction of the cell nuclei are traversed by a particle track.

Abstract: It has been long thought that the important genetic effects of radiation in mammalian cells are the result of direct DNA damage. Thus when cell populations are exposed to very low fluences of alpha-particle radiation, biological effects would occur only in those cells whose nuclei were actually traversed by a track. Presumably, no effects would be expected in the non-traversed 'bystander' cells in the population. We reported earlier (*Cancer Res.* 52, 6394, 1992) that when Chinese hamster ovary (CHO) cell populations were exposed to mean doses where only 1% of the nuclei are directly irradiated, an increased frequency of sister-chromatid exchanges occurred in 30-50% of cells in the population. In the present project, we are focusing on changes in the expression of genes involved in either cell cycle regulation or DNA damage recognition and repair in bystander human cells. Our specific aims are: 1) determine the extent of G1-phase delays under bystander conditions, and correlate these with changes in gene expression; 2) by use of immunofluorescence techniques, examine the in situ patterns of the expression of regulated

proteins, investigate the effects of gap-junction inhibitors on gene expression, and confirm the involvement of intercellular communication in the response to low fluences of alpha-particles through the use of wild-type or connexin43 knockout cells; 3) determine the contribution of reactive oxygen species to the modulation of gene expression; 4) determine the involvement of extra-cellular or membrane originating signaling pathways; and establish whether the inhibition of activated signaling pathways (ATM/p53/p21Waf1) results in lack of enhancement of genetic damage in low-fluence exposed cells. Depending upon the results of the above experiments, we will extend these studies to cell strains derived from individuals with certain genetic disorders and knockout mouse strains for genes implicated in DNA repair (e.g., KU70, KU86).

Investigations are being performed in normal human diploid fibroblasts grown under stringently controlled conditions. Protein expression patterns and induction of genetic damage are being examined primarily by in situ microscopic techniques. Work in progress indicates that the G1 checkpoint is induced in cell populations exposed to mean doses as low as 1 cGy, and is entirely mediated by p53/p21Waf1. We have confirmed the involvement of gap-junction mediated intercellular communication in the regulation of the p53/p21Waf1 pathway, the modulation of expression of cell growth proteins and the induction of DNA damage in non-traversed bystander cells. Preliminary results indicate that the frequency of specific gene mutations may also be elevated in bystander cells. Finally, our data support the involvement of reactive oxygen species in the cellular response to low mean doses of alpha-particles. Overall, our findings indicate that biological effects are not restricted to the response of individual cells to the DNA damage they receive and that cell populations respond as a whole to radiation exposure, with communication occurring among the cells. These results imply that the modeling of dose-response relationships based on the number of cells hit may not be a valid approach.

6. Mechanisms of Tissue Response to Low Dose Ionizing Radiation Exposures: Bioinformatic Tools for Multiparametric Image Analysis

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Summary: This project uses highly sensitive and precise cell biology techniques and newly developed computer-assisted tools to microscopically map complex patterns of radiation-induced proteins.

Abstract: Tissue response to radiation, and hence risk, is a composite of genetic damage, cell loss and induced gene products. We have defined a series of events that occur from 1 hr and 4 weeks after whole body exposure to doses of 0.1 Gy to 5 Gy 60Co-γ radiation in liver and mammary gland, which indicate that remodeling is a general and rapid consequence of irradiation but that the pattern and composition of the radiation-induced ECM is tissue specific. These studies suggest that cell type and interactions play a prominent role in determining the character and composition of radiation-induced remodeling, which in turn represents a

fundamental process initiated by radiation exposure that may promote tissue dysfunction. Alternatively, such events may also be necessary for tissue recovery in a as yet unappreciated way.

Aim 1: Quantitative comparison of radiation-induced changes in ECM composition, growth factor expression and activation, and integrin using immunofluorescence localization as a function of dose (0.1 -5 Gy) and time (1 hr-14 days) of mice from resistant (C57/bl6) and sensitive (Balb/c) genetic backgrounds.

We have analyzed radiation-induced proteins in the C57/bl6 mice that are heterozygotes for the transforming growth factor-β 1 (TGF- β) gene. This strain of mice is known to be relatively resistant to mammary carcinogenesis but sensitive to radiation-induced fibrosis. Interestingly, qualitative assessment of radiation induced proteins such as collagen III and plasminogen activator shows that TGF-β heterozygotes appear similar to Balb/c mice, which are known to be relatively sensitive to mammary carcinogenesis but resistant to radiation-induced fibrosis.

Aim 2: Determine whether low dose radiation exposure affects the ability of human mammary epithelial cells to maintain normal interactions with the microenvironment by studying integrin and E-cadherin expression as a function of dose (0.1 -5 Gy) and time (1 hr-14 days) in 3-dimensional cell culture.

We have examined radiation-induced changes in integrins in HMT-3522 S1 human breast epithelial cells cultured within an extracellular matrix. Confocal microscopy revealed that immunoreactive 6 and 4 integrins localized on the basal surface of are perturbed following γ-radiation exposures of less than 2 Gy. The cells that survive radiation exposure show persistent dysregulation of these integrins and a potential ligand, collagen type IV. TGF-β amplifies this effect.

Aim 3: Develop a bio-informatics framework of integrated image acquisition, annotation, and hierarchical image abstraction (i.e. data model) to create a database that registers localization and intensity information about multiple targets along with positional references and morphological features. Statistical and visualization tools will be integrated to allow hypothesis testing and data mining.

This multidisciplinary approach will result in the construction of phenotypic database of radiation-induced proteins in several animal models and human epithelial cell culture. The identification of critical biological responses to low dose radiation exposure can then be integrated into computational models of radiation risk. Image bio-informatics will also be valuable for functional genomics aspects of the Human Genome Project by providing integrated analysis of complex biological information in the relevant tissue context.

7. Genome Wide Gene Expression of LLIR and Biological Consequences

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Summary: The ultimate goal of this project is the genome-wide identification of genes whose transcription is regulated in response to LLIR and the determination of pathways, particularly involving DNA damage repair and signaling, that are involved in adaptive and bystander effects.

Abstract: The goal of this project is to provide the DOE and the scientific community with an understanding of the molecular mechanisms underlying LLIR exposure and with a means to assess the potential risks of LLIR exposure in humans. This goal is addressed by three specific aims that address issues identified by DOE as being in need of further study. 1) These include the genome-wide identification of genes whose transcription is regulated in response to LLIR. This will provide us with a molecular basis for understanding the process of LLIR exposure as well as specific end points for quantifying if a particular level of exposure may be safe or not. We intend to approach this problem by differentiating between global gene expression patterns induced by low vs high doses of radiation and by identifying genes specific to a particular DNA damage signaling pathway that are modulated by low dose of irradiation. 2) We would also like to identify the genes and signal transduction pathways that mediate a response to LLIR which will help us to understand the mechanism(s) by which LLIR induces an adaptive response in mammalian cells. We will use transgenic mouse cells deficient in specific DNA repair pathways to look at LLIR induced adaptive responses. 3) Finally, we would like to identify the cellular and molecular targets that mediate bystander effects in response to LLIR. We hypothesize that the biological end points of bystander effects are mediated by reactive oxygen species (ROS) and are targeted to DNA directly and plan to identify DNA-damage signaling pathways and potential genes that may mediate these effects. We shall again use transgenic mouse models to identify specific DNA repair genes and pathways that may be involved in induction of bystander effects. Comparisons of global gene expression patterns will help identify potential LLIR or ROS-induced transcripts that may mediate bystander effects.

8. Molecular Mechanisms and Cellular Consequences of Low-Dose Exposure to DNA Damaging Agents

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Summary: This research utilizes cDNA microarrays to characterize the early response of mammalian cells exposed to low doses of ionizing radiation, and to determine whether genes that show modulated transcription after low-dose exposure play pivotal roles in the cell's response to radiation-induced damage and whether variations in response are associated with susceptibilities for cytogenetic damage and risks for tissue pathologies and diseases.

Abstract: Although it is well established that high-dose exposures to ionizing radiation and chemical DNA damaging agents can lead to diverse tissue pathologies and diseases including cancers, there is little molecular understanding of the early cellular response to damaging agents, especially for low-dose exposures. The hypothesis for our research is that genes which show modulated transcription after low-dose exposure to ionizing radiation play pivotal roles in the cell's molecular response to radiation-induced damage and that variations in radiation response are associated with susceptibilities for

cytogenetic damage and risks for tissue pathologies and diseases. This research investigates tissue variation and radiation induced expression modulation among large numbers of genes in parallel utilizing cDNA expression microarrays containing genes known to be involved in DNA repair and inducible after exposure. The initial focus of this work was to: (a) characterize the baseline expression of these genes among normal tissues in vivo and in vitro; (b) identify genes whose expression is modulated early after exposure to low doses of ionizing radiation and compare variations in response among tissues; and © identify modulated genes that are associated with differential risk for cytogenetic damage (adaptive cytogenetic response). cDNAs from genes of interest (called targets) were immobilized onto glass supports using amino modification chemistry which resists strong washings and hybridization procedures. Pools of mRNA from two tissues of interest (called probes) were labeled with different fluorochromes and co-hybridized onto the array. The relative amount of fluorescence at each arrayed spot was used to calculate the differential expression of each gene in the two RNA pools. Our system was validated for specificity, sensitivity and reproducibility of results. For probe concentrations ranging from ~20 nanograms to ~10 picograms, we demonstrated linear responses for up to ~1000-fold differences in fluorescence intensities. Custom image processing software performed automated background subtraction, grid detection, spot detection and quantitation. Synthetic images were used to test system accuracy, and a known dilution series of probes was hybridized onto slides to confirm performance. Tissue variation of DNA repair-related genes was evaluated using cDNA microarrays containing genes involved in double strand break repair, nucleotide excision repair, base excision repair, direct reversal of damage, mismatch repair, cell cycle regulation, apoptosis and damage response. Several differentially expressed genes (up as well as down regulated) were identified in mouse tissue comparisons; research is underway response after low-dose radiation exposure. This ongoing project will provide important mechanistic and molecular knowledge of the cellular and cytogenetic response to low dose irradiation, reduce the uncertainty of assessing risk at low exposure levels, and identify candidate disease susceptibility after low-dose irradiation exposures. [This work was conducted under the auspices of the U.S. DOE by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48]

9. Development of a Functional Genomics Approach Employing Radiation-Induced Changes in Gene Expression to Monitor Cells after Low Dose and Low Dose-Rate Exposures

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Summary: This project explores the utility of gene induction as a means to monitor for exposure to ionizing radiation; molecular biology approaches, including recently developed cDNA chip technology, are employed to study cellular responses to radiation injury.

Abstract: Using a human myeloid tumor cell line (ML-1), we have demonstrated induction of mRNA expression of several stress responsive genes by doses of gamma rays as low as 2 cGy. For instance, the dose-response for induction of CIP1/WAF1 and GADD45 appears to be linear over the range of 2 - 50 cGy, and shows no evidence of a threshold for induction. Although 2 and 5 cGy exposures did not result in any detectable reduction in cloning efficiency or

increased apoptosis in ML-1 cells, these exposures did produce a brief cell cycle delay. We have also used fluorescent cDNA microarray hybridization to investigate transcriptional stress responses following low doses of gamma-rays, and to identify additional radiation-responsive genes for inclusion on a stress-specific microarray we are developing. Using this functional genomics approach, the responses of a substantial portion of a cell's expressed genes can be monitored. As a first step to in vivo studies in humans, the ionizing radiation responses of peripheral blood lymphocytes (PBL) irradiated ex vivo are being characterized. Future studies will be expanded to responses in irradiated mice using the same approaches. Computation-intensive informatics analysis methods are also being developed for management of the complex gene expression profiles resulting from these experiments, and some preliminary results are illustrated. This functional genomics approach should have utility for DOE priorities in radiobiology and toxicology.

Amundson, S.A., Bittner, M., Yidong, C., Trent, J., Meltzer, P., and Fornace, A.J., Jr. Fluorescent cDNA microarray hybridization reveals complexity and heterogeneity of cellular genotoxic stress responses. *Oncogene* 18: 3666-3672, 1999.

Amundson, S. A., Do, K. T., and Fornace, A.J., Jr. Induction of Stress Genes by Low Doses of Gamma Rays. *Radiat Res* 152: 225-231, 1999.

Fornace, A.J., Jr., Amundson, S.A., Bittner, M., Myers, T.G., Weinstein, J.N., Meltzer, P., and Trent, J. The complexity of radiation stress responses: analysis by informatics and functional genomics approaches. *Gene Expression*, 7: 387-400, 1999.

10. Linking Molecular Events to Cellular Responses at Low Dose Exposures

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Summary: Studies are proposed to determine the effect of low dose radiation on molecular events known to be important to carcinogenic processes.

Abstract: Carcinogenesis is considered a multistage process defined on the basis of genetic (initiation) and epigenetic (promotion, progression) mechanisms, yet our understanding of the processes involved is insufficient to address the potential human health effects of low dose radiation. These low dose exposures are important since they reflect the majority of real life exposures and since they are difficult to address experimentally.

The mouse BALB/c JB6 model, which includes several genetic variants for the transformation response, is the best characterized system for examining carcinogenic processes. Most importantly, this model has allowed the linkage of key cell signaling events with cellular response (anchorage-independent growth). By considering the interplay between growth factors and radiation on cell signaling and cellular transformation responses, we have developed a unique system with an unambiguous low dose radiation response, albeit an unexpected inhibition of transformation. Preliminary data suggest the inhibitory effect of low dose radiation on epidermal growth factor

(EGF)-mediated transformation is not cell cycle-dependent. This observation contrasts the EGF receptor-dependent proliferative response observed in certain tumorigenic cell lines exposed to high dose (0.5-5 Gy) radiation. Prior work with the JB6 cell system has demonstrated that several cell signaling pathways act in concert to promote neoplastic transformation. Critical processes include increased oxidative stress, activation of the MAPK pathway, activation of the transcription factor AP-1, and downregulation of p107, a retinoblastoma-related pocket protein. ERK activity in particular appears to exhibit a threshold that is required for the transformation response. We propose to define how these molecular markers respond at low dose exposures (tumor promoter/radiation combinations), relate these responses to clonal growth, and demonstrate their relevance in vivo. This work will develop concepts for cellular responses at low dose exposures and provide the data needed to model those responses at doses below which experimental determinations can be made. We will:

1) Define the effect of low dose radiation on EGF- and TPA-mediated transformation in JB6 cells.
2) Determine whether molecular events known to be critical for transformation exhibit thresholds that are perturbed by low-dose radiation.
3) Determine whether low dose radiation modulates the course of oxidative stress required for cellular transformation in response to tumor promoters.
4) Determine whether the low dose radiation responses demonstrated in vitro are relevant to in vivo processes.

11. Current Cytogenetic Issues Pertaining to Low Dose and Low Dose Rate

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Summary: For both theoretical and pragmatic reasons that impact low dose issues, we propose to study radiation-induced chromosome exchanges in human cells that require three or more breakpoints for their formation, utilizing combinatorial whole chromosome labeling techniques that allow each chromosome to be “painted” a unique color.

Abstract: By providing a quantitative, sensitive and relevant measure of genotoxic damage, the study of chromosomal exchange-type aberrations has served to guide theoretical models of radiation action for half a century. Until recently, it was universally assumed that the vast majority of radiation-induced exchanges between different chromosomes were simple reciprocal events, involving pairs of chromosomes. Newer “chromosome painting” studies suggest that a large fraction of exchange aberrations are actually “complex”, involving three or breakpoints among two or more chromosomes. Putting this discovery in the context of contemporary models of radiation action requires a number of assumptions, because conventional chromosome painting techniques reveal only a partial picture of complex rearrangements. Differing assumptions lead to grossly divergent predictions about the true extent of aberration complexity. This controversy impacts fundamental tenets of radiation action, including prominent generalized theories of radiation action that form the

cornerstone concepts currently applied to issues of radiation protection.

A key aspect of the project is the use of multicolor combinatorial painting (m-FISH) to eliminate discrepancies surrounding the interpretation of complex aberration data. This will allow the testing of two contemporary cytogenetic models. The models chosen are particularly important to the issue of low dose effects, because their predictions have been used to buttress diametrically opposed views of how chromosome aberrations are produced by ionizing radiation. Also described are experiments to examine the feasibility of using complex aberrations as a biomarker of past exposure to densely ionizing radiations.

Specific Aims (abbreviated)

1. Over doses of 137-Cs gamma-rays ranging from 1 to 4 Gy, evaluate the frequency of simple versus complex exchanges in first post-irradiation metaphases of primary human cells. Determine the frequency of total exchanges that are complex (the relative complex fraction; RCF).
2. Measure the frequencies of simple reciprocal and complex aberrations following exposure to 5 Gy of gamma-rays, delivered at the limiting low dose rate of 0.1 cGy/min. Determine the RCF, in order to test the hypothesis that single-tracks from low LET radiations cannot produce complex aberrations.
3. As a function of dose ranging from 0.2 to 1.5 Gy, determine the RCF for 238-Pu alpha particles and for 0.43 MeV neutrons. Test the hypothesis that the RCF is independent of dose for both alpha particles and neutrons, and explore the use of this parameter as a potentially robust cytogenetic biomarker for high LET radiation exposure.

By quantifying the true extent of exchange complexity, m-FISH allows for the testing of two models used to buttress diametrically opposed mechanisms of aberration formation. There are two key issues at stake for the Low Dose Program. The first is whether complex exchanges display a near-quadratic dependency on dose, as this implies a “virtual threshold” for their formation at low doses. Secondly, the model chosen will dramatically affect estimates for low-dose yields of simple exchanges. The development of a cytogenetic “signature” of prior high LET exposure would have far-reaching implications relating to radiation quality factors used in risk management.

12. Adaptive Response Against Spontaneous Neoplastic Transformation In Vitro Induced by Ionizing Radiation

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Summary: The goal of this project is to determine if repeated low doses of gamma radiation can induce an adaptive response against neoplastic transformation and thus address the question of the validity of linear extrapolation of high radiation dose data to estimate low dose risk.

Abstract: Adaptive responses induced by low doses of radiation have typically been detected by observing a reduced response to a subsequent challenge with a high radiation dose. For the endpoint of neoplastic transformation there are two reports which have been able to detect an adaptive response without using the high dose challenge approach (Azzam et al., *Radiat. Res.* 146: 369-373,1996; Redpath and Antoniono, *Radiat. Res.* 149: 517-520, 1998). These reports compared the transformation frequency induced by low single doses (<10 cGy) with the spontaneous background frequency seen in sham-irradiated cells and found that the frequency was reduced in the low dose irradiated group.

The specific aims of the current project are:

1. To extend the dose range of a previous study using a single dose of 1 cGy to include 0.1 and 10.0 cGy. In addition, multiple exposure treatment regimens (e.g. 10 x 0.1 cGy) will be used.
2. To examine the possible role of repair in the adaptive response.
3. To compare the efficacy of Cs137 gamma radiation and 80 kVp x-radiation in inducing an adaptive response.

The studies will be performed using the HeLa x skin fibroblast human hybrid cell transformation assay system. A single batch and lot of serum will be used for all studies to eliminate any influence of this possible variable. All control, unirradiated groups will undergo a sham-irradiation. A post-irradiation holding time of 24 h for induction of the adaptive response will be used. To examine the possible role of repair in the adaptive response, experiments will be performed where post-irradiation holding at 37ºC and 22ºC will be compared. Previous work with this assay system has suggested that holding at 22ºC promotes misrepair (Redpath and Antoniono, *Radiat. Res.* 144: 102-106, 1995). All experiments will be repeated six times. Poisson regression will be used as the general framework to analyze and evaluate the data.

It is anticipated that at the completion of the proposed studies a definitive answer to the following questions should be provided:

1. Is there an induction of an adaptive response against spontaneous neoplastic transformation in vitro by repeated exposure to low doses of ionizing radiation?
2. Does extrapolation from high dose data accurately predict the induced frequencies at low doses?
3. Does promotion of misrepair abrogate any adaptive response?
4. Are 80 kVp x rays more or less effective than Cs137 gamma rays at inducing an adaptive response?

13. Role of the "Adaptive Response" in Determining Health Risks from in Vivo Exposures to Low Doses of Ionizing Radiation

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Summary: This investigation will determine whether low doses of ionizing radiation administered to cultured cells and to rodents will induce cellular and molecular alterations which affect the responses to subsequent radiation exposures, as estimated by the level of chromosome damage.

Abstract: The phenomenon known as adaptive response is the ability of a low "priming" dose of radiation (usually < 10 cGy) to ameliorate the adverse effects of a subsequent "challenge" dose (1-2 Gy). Many human exposures involve

uneven dose rates, raising the possibility that recent radiation exposure history and therefore the adaptive response phenomenon may be important for determining the biological consequences of subsequent exposures. Cancer is the outcome of greatest concern to individuals occupationally or accidentally exposed to low doses of ionizing radiation (IR), yet little is known about the cytogenetic, cellular and molecular responses to low dose IR.

Our specific aim is to verify that the adaptive response exposure regime results in amelioration of damage from in vivo IR exposure using molecular cytogenetics as the endpoint. To accomplish this, we will characterize in vivo adaptive responses to low IR doses by exposing rats to a small acute "priming" dose of 137Cs gamma radiation (<5 cGy) followed by an acute "challenge" dose of 137Cs (0.5 to 4 Gy). Control animals will receive no radiation, or the priming or challenge dose alone. We will determine the dose/time treatment regime that produces optimal adaptation in peripheral lymphocytes after the second dose using chromosome aberrations by painting. Preliminary in vitro experiments will address the same question plus determine the role of strain differences. Insight into gene and protein responses to differing levels of low-dose IR will also be obtained.

Several aspects of the data to be obtained from these studies should provide a basis for a re-evaluation of the current regulatory guidelines for exposure to ionizing radiation. These guidelines and regulations on exposure limit have a direct impact on strategies for remediation of waste sites that are the responsibility of DOE Environmental Management. Our results are expected to facilitate cost reductions in cleaning up DOE facilities because improved risk calculations will increase the precision and decrease the uncertainties in estimating the resources required for environmental remediation.

14. Genomic Instability Induced by Low Dose Irradiation

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Summary: The induction of genomic instability by low doses of ionizing radiation will be determined by measuring the delayed increase in mutation frequency in a target gene that requires a frameshift mutation for the easily and directly quantifiable expression of the green fluorescent protein.

Abstract: The objective of the research described in this application is to determine the dose response of the induction of genomic instability by low doses of ionizing radiation, as indicated by a delayed increase in mutation frequency. The delayed increase in mutant frequency will be measured in stable human colon carcinoma cell lines transfected with a vector carrying a reporter green fluorescent protein (GFP) that requires a frame shift mutation for activation. Expression of GFP results in quantifiable colored cells. Because the delayed increase in mutant frequency caused by the induction of genomic instability can be visualized and quantified directly in cells surviving irradiation, it can be detected quickly among many thousands of transfected cells. Such

a strategy is necessary for the quantification and investigation of the induction of genomic instability by low doses of ionizing radiation. In addition, the approach can facilitate distinction between the immediate and delayed effects of exposure to ionizing radiation. The induction of genomic instability is relevant with regard to risk estimation since it is thought to be a necessary step in the carcinogenic process.

The hypothesis driving the research is that the induction of genomic instability leading to an delayed increase in the frequency of gene mutations many generations after exposure to radiation can be initiated by radiation-induced, complex, poorly repaired DNA damage that leads to a mutator phenotype. We aim to (1) measure the dose response of the induction of genomic instability by Cs gamma radiation with doses ranging from 0.05 Gy to 3 Gy in order to determine if there is a dose-dependent induction of genomic instability and if there is a threshold apparent in the dose response curve; (2) measure the kinetics of the induction and termination of genomic instability at intervals following exposure to irradiation; (3) compare the induction of genomic instability caused by hydrogen peroxide with that caused by radiation to determine whether or not clustered lesions are required for the induction of genomic instability; (4) measure the dose-response of the induction of genomic instability in cell lines varying in their genetic background with regard to mismatch repair capacity, and the activity of the p53 and p21 genes; and (5) isolate and characterize the unstable cell lines in order to investigate the initial lesion inducing the instability.

The characterization of the induction by low doses of radiation of genomic instability, a process that is thought to be necessary for the carcinogenic process, will be helpful in the estimation of the risk of cancer in persons exposed to these low radiation doses.

15. Development of PCR Based Methods for the Detection of Mutagenic Effects of Ionizing Radiation

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Summary: The aim of this project is to devise a series of sensitive polymerase chain reaction (PCR) based methods for detecting three different molecular classes of mutation known to be caused by radiation and then study how the frequency of these events changes when cells are exposed to low doses of ionizing radiation.

Background: Mutation detection in human cells commonly employs phenotypic selection which has limitations for detecting low doses of mutagens. Phenotypic selection has the further disadvantage that it can only be applied to a limited range of tissues. Furthermore ionizing radiation causes different classes of mutations at the molecular level. These can be grouped into small intragenic events (point mutations and minor deletions), large deletions and major chromosomal rearrangements including mitotic recombination, translocation and inversions.

In order to study the genotoxic effects of low dose radiation it is desirable to have available techniques which permit the sensitive detection of mutation by molecular class and in a wide range of tissues.

Specific Aims:

- The development of novel PCR based techniques for the enhanced detection of point mutation, major deletion and inversion events in DNA.

- The determination of dose-response curves for mutations in each of these molecular classes arising from low-dose and -dose rate ionizing radiation.

Summary of Research Approach: Point mutations will be detected in target sequences. A number of existing strategies will be employed with modifications that will enhance sensitivity of detection and minimize artefactual induction of mutation by polymerase. These will be combined with a novel method in which there will be enrichment of the target sequence of interest prior to PCR. Subsequent PCR with high fidelity polymerase, heat stable restriction enzymes and the use of PNA inhibitors of non-target sequences is expected to permit the detection of rare mutational events against a background of wild-type sequences.

Techniques for detecting deletions and inversions will be based on modifications of existing protocols. These have been demonstrated to detect rare mutations but their sensitivity for the detection of low dose radiation damage remains to be established.

Anticipated Scientific Benefits: The outcomes of this project are expected to add to information about the mutagenicity of low dose radiation in two important ways. Firstly it will provide techniques which will allow the burden of mutations to be measured in any accessible tissue. This will permit tissues to be identified which may have differential sensitivities to radiation, and perhaps identify those in which mutations better reflect the accumulation of genotoxic damage. Secondly, through measurement of how the different classes of mutation are generated in response to dose and dose rate, we may learn more about the basic molecular events which are the important genotoxic ones at low dose radiation.

16. Low Dose, Low Dose Rate Effects of Ionizing Radiation in Irradiated and Unirradiated Cells

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Summary: The goals of this project are to understand the mechanisms by which effects of ionizing radiation (IR) can occur in cells that are not directly irradiated and to comparatively determine the consequences these so-called “bystander” effects may play in the context of human risk due to exposure to low level ionizing radiation (LLIR).

Abstract: Particular emphasis is given to targeted and nontargeted processes that involve the generation of reactive oxygen species (ROS) because of the roles ROS may play in mediating DNA damage, cell cycle-related effects, and genomic instability. The specific aims of this project are to: 1) comparatively determine how the intracellular ROS increases and the associated “bystander” responses to low dose, high-LET a particles we have recently discovered may extend to low dose/low dose rate exposures to low-LET IR and further assess the biological consequences of the responses, 2) comparatively assess the extent, quality, and repair of DNA damage induced by basal oxidative metabolism and low dose/low dose rate IR in the presence and absence of: a) IR-induced increases in cell-mediated ROS production, and b) IR-associated intracellular

ROS increases that occur as a bystander effect, 3) elucidate the molecular and biochemical pathways that underlie ROS and cell cycle responses to LLIR in irradiated and nontargeted cells, and 4) determine how the ROS and cell cycle responses in irradiated and bystander cells affect cell growth, radioresistance, and genomic stability following subsequent bouts of exposure to IR. The project will provide new information about relationships between LLIR and the generation of potentially damaging ROS, how DNA damage and repair that occurs during normal oxidative metabolism compares with that which may occur with LLIR, adaptive responses to LLIR, cell proliferative and cell cycle responses to LLIR, and “bystander” effects, including genomic instability, that may occur following exposure to LLIR. Moreover, an understanding of bystander mechanisms may lead to the identification of genes/gene products that figure into responses to LLIR, which ultimately may be useful for identifying polymorphisms that render individuals more or less susceptible to the effects of LLIR.

17. Gene Targeting and Nonhomologous End-Joining in *Saccharomyces*

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Summary: Model system in *Saccharomyces cerevisiae* for studying gene targeting and nonhomologous end-joining.

Abstract: The repair of double-strand breaks (DSBs) on chromosomes can occur by both homologous and nonhomologous recombination. Nonhomologous DNA end-joining (NHEJ) in budding yeast is carried out by DNA ligase 4, and also requires the accessory protein, Xrcc4, the Ku70 and Ku80 proteins, and the Mre11/Rad50/Xrs2 complex.

1. We have shown that the strong effects on NHEJ of deleting genes encoding Sir2, Sir3 or Sir4 is mostly a consequence of changing the expression of mating-type genes; however a small direct role of Sir proteins on NHEJ is seen, when mating phenotypes are not affected. We also find that mating type regulates the balance between homologous and nonhomologous recombination. In collaboration with Audrey Gasch and Pat Brown (Stanford) we have identified about a dozen uncharacterized mating type-regulated genes that may play a role in shifting the balance between NHEJ and homologous recombination. These are being analyzed.

2. The role of Mre11p in NHEJ has been further characterized, in collaboration with Debra Bressan and John Petrini (Univ. Wisconsin, Madison). Among 4 site-directed mutations in evolutionarily conserved residues of Mre11p, the severity of defect in NHEJ correlates with their defects in 5' to 3' resection of DSB ends, which in turn correlates with the ability of the mutants to form a complex with Rad50p. Over-expression of the 5' to 3' exonuclease, Exo1p, partially complements the defect of deleting Mre11p, but artificial resection of DNA ends in vitro does not substitute for Mre11's function.

3. The balance between accurate gene targeting and nonhomologous insertions is being studied. The effect of mutations that reduce NHEJ are being examined to see if they change the ratio of targeted to non-targeted transformation, using transforming DNA with very limited homology to the targeted site. In addition we are studying “hit and run” transformation, whereby a transforming DNA fragment initially interacts with a homologous target site, initiates new DNA synthesis from one end of the fragment, but then it dissociates from its template and inserts elsewhere in the genome.

18. Mechanisms of DNA Damage Responses to Low Dose Ionizing Radiation: Molecular, Biochemical, and Cellular Studies

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Summary: Identification and characterization of mechanisms and gene products involved in three different crucial pathways for repair of DNA double-strand breaks and other oxidative lesions.

Abstract: Although the importance of genomic surveillance pathways for avoidance of carcinogenesis is clear, the detailed nature and regulation of DNA repair processes responsive to low level ionizing radiation (LLIR) is still largely unknown. The possibility that repair pathways are induced by LLIR is of particular significance for assessment of risk from environmentally relevant doses. For example, failure of low doses to induce repair systems might cause disproportionate genetic damage, whereas continuous exposure to LLIR might be protective relative to risk estimated from linearity. It is therefore essential to critically examine the detailed mechanisms and the regulation of repair systems for LLIR-induced damage. In particular, DNA double-strand breaks (DSBs) produced by IR are presumed to account for its clastogenic effects as well as its high lethality. Thus efficient correct rejoining of DSBs is essential for maintenance of genomic integrity, and cells deficient in DSB rejoining are hypersensitive to genetic damage by LLIR. In addition, a transcription-coupled base excision repair (BER) pathway is critical for mutation avoidance and is implicated in LLIR-inducible BER at oxidative lesions. These two pathways may be interconnected or jointly regulated in response to LLIR. This research program is designed to identify and characterize the proteins and pathways involved in repair of DSBs and base damage induced by LLIR, their associated damage sensing pathways, and the protein-protein interactions critical for their function through three specific aims: (1) to identify and characterize the components of multi-protein complexes for DNA damage sensing and double-strand break repair (DSBR) after LLIR; (2) to investigate cellular parameters affecting the efficiency and fidelity of DSBR at very low levels of damage, including possible inducibility and overlap with transcription-coupled repair (TCR); and (3) to characterize LLIR-inducible base excision repair (BER) and its relationship to the adaptive response, the bystander effect, and both spontaneous and LLIR-induced mutagenesis. Our approach thus includes closely coordinated genetic, molecular and biochemical analyses and in addition employs ultra-sensitive assays for damage detection. These studies will also create the foundation for structure-function analyses of critical repair proteins.

19. Assessing Biological Function of DNA-Damage Response Genes

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Summary: Our research utilizes rodent and human cell lines grown in culture to identify the molecular components of DNA repair pathways that act on radiation damage and to determine how these pathways are regulated in a way that minimizes mutations and genetic instability, which can lead to cancer.

Approach: Although many genes that likely participate in repairing DNA damage produced by ionizing radiation (IR) are identified, often the quantitative biological contribution remains unknown. Moreover, the processes that regulate and coordinate DNA repair in the context of the cell cycle are poorly understood. At low doses (< 25 cGy) where there almost no killing, cells may experience reproductive or apoptotic (genetically programmed) cell death, survive with or without deleterious mutations, or, presumably, enter a genetically unstable state that constitutes a major pathway toward cancer. This instability is associated with increased reactive oxygen species (ROS). The goal of this project is to use the tools of molecular genetics to define the cellular responses to IR damage that minimize the occurrence of gene mutations and genetic instability in surviving cells. We will determine the relative contributions of different DNA repair pathways in coping with IR damage. These pathways are base-excision repair (for oxidative base damage and single-strand breaks), nonhomologous end joining (NHEJ) of double-strand breaks (DSBs), and homologous recombinational repair (HRR) of DSBs. One major focus is the HRR pathway, which (unlike NHEJ) presumably repairs DSBs without making mutations and plays a more significant role than previously realized. Our central experimental premise is that the construction and systematic characterization of mutants is necessary to quantify the biological impact or risk of low doses of IR. Through "gene targeting" methods, cell lines mutated in genes likely to be important in low-dose responses will be constructed and characterized. Hamster CHO cells are used because of their fast growth rate, stable chromosome number, established gene targeting capability, and a historical database of IR studies. The gene knockout mutants will be characterized to determine the contribution of each gene and pathway to low-dose IR exposure. The consequences of the mutations will be evaluated in terms of increased cellular susceptibility to chromosome damage and single-gene mutation, and altered apoptosis, cell-cycle sensitivity, and cell-cycle progression. In addition to identifying components of the DNA repair machinery, we have uncovered what may be to be an important connection between genes affecting radiation sensitivity and IR-induced genomic instability. Identification of a gene (FANCG/XRCC9) involved in the cancer-prone disorder Fanconi anemia (FA) has lead us to hypothesize that the complex FA multi-gene homeostasis pathway may be closely related to IR-induced genomic instability. To test this hypothesis, we plan to characterize human lymphoblasts from FA group G patients compared with their gene-complemented derivatives.

Deliverables: This work will produce a much better understanding of the relative importance of the different DNA repair pathways in cellular resistance to low-dose IR exposure. Knowledge of FA protein function should help clarify the mechanism of genomic instability. Unrestricted international distribution of the CHO mutants to other laboratories will greatly augment the mutants' value through collaborative or independent studies. The mutants will also be very suitable for evaluating the potential dysfunction associated with human genetic variants of DNA repair genes that are being detected at high frequency (e.g. 1-10%) in the general population under DOE funding.

20. The Dynamic Behavior of Broken Chromosomes in Yeast

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Summary: We will observe the movements of both ends of a broken chromosome in yeast to determine the functions of previously identified DNA repair genes.

Abstract: Two major DNA double-strand break (DSB) repair pathways, homologous recombination and nonhomologous end joining, have been characterized in both the budding yeast *Saccharomyces cerevisiae* and in humans. A significant number of proteins have been identified whose functions within these pathways are necessary for the repair of DNA double strand breaks. The requirement for each of these proteins at specific steps of the DNA repair process has yet to be determined. We propose to use yeast as a model system to study the dynamics of chromosomes as they undergo breakage and repair. Initial studies will examine the cytological fate of both ends of a single chromosomal DSB induced by HO endonuclease using video imaging of fluorescently tagged chromosomal DNA in living cells. The fate(s) of both ends will be followed in haploid and diploid cells in both G1 and G2 to assess the impact of increased or decreased opportunities for DSB repair by homologous recombination. Real time analysis of the metabolism of a broken chromosome will be performed in mutant strains that are defective in both major pathways of DSB repair as well as for strains containing a deletion of the checkpoint gene RAD9. Our progress thus far includes the successful construction of a chromosome marker cassette that can be integrated into the yeast genome at any desired location and confirmation that movements of a chromosome containing this marker can be easily followed by microscopic imaging system. We have currently integrated this marker at either side of HO cut sites located on two different chromosomes. We are also in the process of developing a system utilizing different spectral variants of green fluorescent protein, allowing multiple structures or multiple chromosome markers to be easily imaged in the same cell. We have also examined the effect of a deletion of the yeast homologue of the nonhomologous end joining protein Ku70 on repair of dicentric chromosome breakage and have found that a loss of Ku results in a loss of chromatin structure between the dicentric centromeres.

Given the number of yeast DNA repair genes that have human structural homologues we anticipate our findings in this project will identify specific roles of genes necessary for the repair of DNA damage that results from exposure to low dose radiation.

21. Repair of DNA Damaged by Ionizing Radiation and Other Oxidative Agents in Yeast and Human

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Summary: The hallmark of oxidative damage produced in DNA by ionizing radiation and hydrogen peroxide is DNA strand breaks with blocked ends. How this damage is repaired, and what happens to cells if it is not repaired will be determined in yeast and human cells.

Abstract: Treatment of cells with oxidative DNA damaging agents such as ionizing radiation

and H_2O_2 produces .OH radicals which attack DNA, producing single and double strand breaks that have a phosphoglycolate at the 3'-terminus. While DNA strand breaks with 3'-blocked termini are the hallmark of oxidative DNA damage, the mechanisms by which such blocked 3'-termini are removed in eukaryotes remain poorly understood. Our goals are to identify the various genes that function in cleaning the blocked 3'-ends from DNA strand breaks generated by treatments with ionizing radiation and H_2O_2, and to determine the biological consequences when such damage is not repaired. Because of the high degree of conservation of DNA repair proteins between yeast and humans, and because of the ease of genetic manipulations, initial studies will be carried out in the yeast *Saccharomyces cerevisiae*. The homologous genes and proteins will then be studied in humans.

Our studies indicate that the yeast APN1 and APN2 genes provide alternate pathways for the repair of single strand breaks with 3'-blocked termini. The apn1D apn2D mutant is highly sensitive to H_2O_2, and the repair of single strand DNA breaks with 3'-blocked termini is greatly reduced in the apn1D apn2D mutant. We will determine the role of the APN1 and APN2 genes in the repair of single strand breaks induced by gamma-ray irradiation. The biological consequences of the 3'-blocked ends, when they are not removed, will be assessed by examining the incidence of mutations and recombination in the apn1D apn2D strain treated with H_2O_2 or gamma rays. New genes and proteins that function with Apn1 and Apn2 will be identified by the yeast two hybrid method and by biochemical means.

The human APN1 gene will be cloned and its role in DNA repair examined in mice. As additional yeast genes that function with Apn1 or Apn2 are identified, their human counterparts from humans will be isolated and studied. The enzymes that function in the removal of blocked 3'-ends from DNA double strand breaks will be identified. One strong candidate for such a role is the Mre11 protein, which has a 3' → 5' exonuclease activity, and which exists in yeast as a complex of Mre11-Rad50-Xrs2 proteins. The ability of the yeast and human Mre11 complexes to remove 3'-blocked termini from DNA substrates will be determined. This study should enhance our understanding of the Nijmegen breakage syndrome in which patients suffer from increased cancer incidence, and which results from a defect in one of the subunits of the human Mre11 complex.

In order to understand the significance of genetic polymorphisms in human DNA repair genes which may affect susceptibility to low dose radiation and to other oxidative DNA damage, it is imperative to first identify the various genes that function in the repair of such damage. Because of its genetic versatility, these studies will first be done in *S. cerevisiae*. The information derived from yeast studies will be used to identify the human counterparts of these genes and to study their function in DNA repair.

Radiation vs Endogenous Damage

22. Biological Effects of Low Level Ionizing Radiation and Normal Oxidative Damage: The Same or Different?

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Summary: This project will determine whether genetic damage caused by low level ionizing radiation is similar to, or different from, incidental oxidative damage caused as a byproduct of normal metabolic processes.

Background: Low LET radiation produces most of its effects through the generation of reactive oxygen species (ROS). These ROS are not unique to radiation exposure, but are also produced continuously during the course of aerobic metabolism. In vitro radiobiological experiments are conducted almost exclusively under normal atmospheric oxygen, a value that exceeds the in vivo physiological oxygen concentration by about six fold at sea level. Thus the true cellular response to low level ionizing radiation (LLIR) may be masked or distorted by an unnaturally high level of oxidative lesions imposed by non-physiological O_2.

Specific Aims: It is the goal of this newly funded project to examine genetic damage, and cellular responses to that damage, induced by LLIR, non-physiological O_2, or a combination of the two. These data are required to test the hypothesis that LLIR produces biological effects that are fundamentally different from those caused by endogenous oxidative damage. Data in support of the hypothesis would provide a firm rationale for radiation protection concepts that seek to limit radiation exposure to the lowest reasonably achievable level. Alternatively, genetic damage induced by LLIR may be essentially the same as endogenous oxidative damage. If so, then LLIR would impose a small, and often temporary, increase in the overall burden of genetic damage. This condition would not differ qualitatively from variations in endogenous oxidative damage that occur naturally in response to stimuli such as exercise, dietary change, or infection. In this case radiation protection standards for LLIR might be relaxed.

Research Approach: We are establishing a laboratory capable of performing in vitro experimentation over a wide range of oxygen partial pressures (from 2.5 to 95% O_2). This 38-fold difference will result in dissolved O_2 concentrations ranging from slightly hypoxic, through physiological (4.3% O_2 at the elevation of Los Alamos), to “normal” atmospheric cell culture conditions (19% O_2), and on to clearly hyperoxic. Taking the physiological O_2 concentration as the appropriate control condition, we will examine and compare the consequences of inducing genetic damage either

by LLIR or temporary exposure to elevated O_2. We will determine if transient oxidative stress induces the same genetic effects, such as DNA base damage, chromosome aberrations, HPRT mutation, and transformation, as exposure to ionizing radiation. Furthermore, we will determine if important cellular responses, such as bystander effects, the adaptive response, and genomic instability, can be induced by LLIR without the additional oxidative

Anticipated Scientific Deliverables: The work will determine whether, and to what extent, oxygen concentration affects radiobiological studies, and will shed light on the question of whether the biological effects of LLIR and normal oxidative damage are the same or different.

23. Free Radical DNA Damage Produced Endogenously and by Low Dose Radiation in Human Cells: Quantitation, Consequences and Repair

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Summary: The overall goal of the proposed studies is to elucidate the relationships between endogenous free radical DNA damage and its repair with the free radical DNA damage produced by low doses of ionizing radiation.

Abstract: Specifically, the proposed studies will quantify, using capillary electrophoresis coupled with laser-induced fluorescence detection, the number of specific radiation-induced free radical lesions produced at very low doses (0.01 - 0.1 Gy) over and above the background level of endogenous lesions. The role that attempted repair of multiply damaged sites, clustered lesions uniquely produced by ionizing radiation, plays in the production of double strand breaks, will be addressed. Whether or not there is a threshold for the repair of radiation-induced free radical DNA lesions will be determined and the relationships between unrepaired lesions to biological endpoints such as lethality and mutagenesis ascertained. Finally, whether or not the enzymes that repair free radical-induced DNA damage are induced by low levels of ionizing radiation will be assessed, and the relationship between this induction and adaptation to mutation production/lethality caused by challenge doses of ionizing radiation will be determined. These experiments address four specific key questions in the DOE Low Dose Radiation Research Program and are aimed at quantifying the production and repair of free radical-induced DNA damage in the background of the same endogenously produced lesions, information essential for determining risk estimates at very low doses.

24. DNA Damage Clusters in Low Level Radiation Responses of Human Cells

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Summary: This program focuses on determining the role of clustered DNA damages—two or more damaged sites within a few DNA helical turns—in human cells exposed to low doses/low dose rates of ionizing radiation.

Background: Clustered DNA damages (two or more closely spaced lesions on opposing strands–including abasic site clusters, oxidized purine clusters, oxidized pyrimidine clusters and

double strand breaks) are hypothesized to be poorly-repaired lesions producing cytotoxic and mutagenic effects of ionizing radiation. However, because no methods existed for quantifying clustered damages in genomic DNA, it was not known if they are induced at low doses, or their impact, if they are produced.

We have developed methods for quantifying clustered damages in genomic DNA, including that in human cells. Our initial data show that clustered damages are produced by a single radiation hit mechanism, and thus that they can be induced at low radiation doses. Further, they are induced in human cells irradiated with low doses of gamma rays.

Specific Aims:

1. Determine whether clustered damages induced endogenously in human cells by normal cellular oxidative metabolism.
2. Measure the dose-response function for induction of clustered damages containing oxidized bases or abasic sites in mammalian cells, and determine what factors affect the response.
3. Delineate cellular repair mechanisms for dealing with clustered damages, their effectiveness and the effect of up- or down-regulation of oxidative base excision repair (BER) enzymes.
4. Determine whether the initial or persistent levels of specific classes of clustered damages are molecular signatures of ionizing radiation exposure.

Research Approach: Clustered DNA damages will be measured using methods we developed previously for quantifying isolated DNA damages: DNA isolation, treatment of DNA with lesion-specific enzymes and agarose gel electrophoresis along with molecular length standard DNAs. Quantitative electronic imaging (developed at BNL by J. Sutherland) will be used to obtain DNA profiles for all experimental samples. The frequencies of damages will be quantified using number average length analysis by the method of moments.

Anticipated Scientific Deliverables:

1. Establish a robust scientific basis for evaluating the effects at the molecular level of low dose/low dose rates of low LET radiation on human cells.
2. Provide practical criteria and methodology for assessing radiation exposure to humans and to organisms in the environment.

25. Micronutrient Deficiency as a Radiation Mimic

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Summary: Dietary micronutrient deficiencies appear to mimic radiation and we wish to compare them to radiation.

Abstract: Folate deficiency breaks chromosomes[1] due to massive incorporation of uracil in human DNA (4 million/cell) with subsequent single strand breaks in DNA formed during base excision repair: two nearby single strand breaks on opposite strands cause the chromosome to fall apart. The level of folate where we see high uracil and breaks was present in 10% of the U.S. population and a much higher

percentage of the poor. Vitamin B12 (14% elderly) and B6 (10% of U.S.) deficiencies also cause high uracil in human DNA and chromosome breaks as indicated by our new evidence and as expected from mechanistic considerations. Many micronutrient deficiencies are likely to damage DNA by the same mechanism as radiation, by causing single- and double-strand breaks, oxidative lesions, or both[2], appear to be orders of magnitude more important, and should be compared for perspective[1;2]. In addition, endogenous oxidative DNA damage is appreciable and increases with age[3;4].

Our aim is to compare low dose radiation with micronutrient deficiency, and endogenous damage, by a variety of measures of DNA damage. Our assays include oxo8Gua, the comet assay, a new FacSort assay for micronuclei, a new sensitive ELISA assay for AP sites in DNA, and DNA microarrays for DNA damage and oxidative damage genes, as well as a very sensitive and specific GC-MS assay for lipid peroxidation. We plan to use IMR90 human diploid fibroblast primary cells in some of the initial work, comparing micronutrient deficient and sufficient cells, and with varying levels of radiation.

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Tool Development

26. Low-Dose Studies with Focused X-rays in Cell and Tissue Models: Mechanisms of Bystander and Genomic Instability Responses

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Summary: The project uses techniques developed to irradiate individual cells in culture, or subcellular regions, to determine mechanisms by which they are damaged by extremely low doses of radiation and whether there are threshold-type responses related to the pathways by which cells respond to oxidative damage that occurs as a byproduct of their metabolism.

Abstract: For low-LET radiations, the concept of DNA damage leading to mutation and cell death has been widely accepted, particularly at high doses (>1 Gy). However, this view has been brought into question with accumulating evidence that a number of transmitted effects play significant roles in radiation responses. The aim of this study is to determine the roles of inter- and intra-cellular signaling in responses relevant to cancer induction by low-LET radiations. The study utilizes a unique focused low-energy X-ray microprobe which we have developed. This provides the ability to target regions of one or more cells selected within a population using a ~0.5-micron diameter beam of low-energy X-rays and revisit the irradiated and unirradiated cells and their progeny in time-lapse fashion to score effects on a cell-by-cell basis. Single-cell irradiation studies with our charged-particle microbeam have shown unequivocal evidence for bystander responses in unirradiated cells and a dose-effect relationship that indicates a low-dose threshold. The project, which uses the focused X-ray microprobe, extends these studies to low-LET, targeting individual cells within populations with the capability of searching down to ~0.1 mGy for the low-dose threshold. In particular, we will use this approach as a paradigm of the ultimate low-LET low-dose exposure of a cell, the action of a single electron track directed at a known sub-cellular target. The project builds on our experience of oxidatively induced cell signaling involved in apoptotic and related responses to examine whether radiation-induced responses and dose-effect thresholds are influenced by oxidative damage levels. These studies will include observation of both early and delayed responses to radiation and oxidative treatments to determine mechanisms involved in the induction of genomic instability.

We will test whether direct radiation exposure of the nucleus and damage to nuclear DNA is required to induce the instability phenotype and/or bystander effects. Non-nuclear targets may be important, for example mitochondria, as they are key players in handling oxidative activity within cells and are central to the apoptotic pathway. The role of damage-sensing pathways, such as p53, perturbations in progression through the cell cycle and the levels

of oxidative activity in hit and non-hit cells will be important measures of the propagation of effects both within and between cells. The involvement of gap-junctional intercellular communication (GJIC) at low doses versus mechanisms involving reactive oxygen species in the medium will be tested. We will test the combination of increased oxidative stress (induced by peroxides and thiols) with targeted X-rays to determine the interactions between the basal levels of oxidative activity in cells and any radiation-induced signaling and stress mechanisms.

In summary, the studies are based on our experience of signaling responses induced both oxidatively and by radiation, seeking mechanistic relationships between them that may contribute to the development of improved models of human radiation risk. Using our novel low-energy X-ray microprobe and cytometry facility, the emphasis is on studying the low doses and isolated electron track type of exposure generally occurring at the cellular level in people exposed to protection-level doses and dose rates of low-LET radiation.

27. Markers of the Low Dose Radiation Response

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Summary: The goal of this work is to develop a technology that will allow direct visualization of DNA double-strand break repair complexes in their original places in the nuclei of irradiated cells.

Background: Ionizing radiation has a unique ability to induce damage simultaneously at multiple sites within a spatially restricted region of DNA. The resulting double-strand DNA breaks (DSBs) present a major threat to the integrity and stability of the genome. Our understanding of the origin and fate of DSBs is based primarily on studies at high radiation doses. Despite recent progress toward the elucidation of molecular mechanisms underlying DSB repair, technologies are still not available to visualize individual DSBs and DSB repair complexes in situ, in irradiated cells.

Specific aims: This project will involve development of a technology to allow direct in situ visualization of DSB repair complexes. These studies will help bridge the gap between biochemical studies of repair enzymes and an understanding of the process of repair as it actually occurs within the radiation-injured cell. Specific aims include:

1. The development of tools for in situ visualization of DSB repair complexes.
2. The use of these tools in combination with fluorescence microscopy to detect and characterize visible subnuclear structures associated with DSB repair.
3. A demonstration of the practical utility of the tools and assays developed in Aims 1 and 2 by quantitative studies of DSB repair in different cell types.

Summary of key research approach: Previous knowledge of the biochemistry of DSB repair will facilitate the development of tools to allow visualization of the active, assembled form of DSB repair proteins. These tools will include fluorescently tagged repair proteins that can be used to visualize repair complex assembly in living cells. They will also include recombinant single-chain antibodies that will be used to detect changes in the conformation and phosphor-ylation state of DSB repair proteins that accompany the assembly of the repair complex. After suitable tools have been developed, control and reconstruction experiments will be performed to correlate visible structures with

biochemically-defined repair complexes. Strategies will be developed to prolong the lifetime of repair complexes to make them more easily visible. Immunoprecipitation experiments will be performed to identify additional proteins that contribute to formation of repair structures. The formation of repair complexes at high and low radiation doses will be compared. The effect of a low radiation dose on the response to subsequent, higher doses will be determined. The relative prevalence of the Ku-dependent end joining and Rad51-dependent recombinational pathways will be investigated.

Anticipated scientific deliverables: The ability to visualize single DSB repair complexes formed at the sites of DNA damage will provide an improvement of two orders of magnitude in sensitivity over existing physical methods of measuring double-strand breaks. It will open up exciting prospects for direct investigation of the low-dose radiation response.

28. A Novel, Spatially Resolved Cell Irradiator to Study Bystander and Adaptive Responses to Low-LET Radiation

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Summary: The goal of this program is to deliver mechanistic information concerning the biological consequences associated with low doses of low-LET radiation at the level of understanding required for the development and implementation of safe and defensible radioactive waste treatment and storage strategies.

Abstract: Currently, standards for human exposure to ionizing radiation are set by extrapolating data obtained in the high-dose regime to the low-dose regime where biological responses are below standard detection limits and capabilities. This program will provide quantitative data and a molecular level understanding of the cellular responses, biological consequences and health effects associated with low-doses of low-LET radiation. Specifically, we will I) develop a low-cost device for spatially resolved and energy controlled electron irradiation of live cells, ii) investigate mechanisms of cell bystander effects and, iii) investigate early steps in pathways leading to adaptive responses.

An initial stage of this program focuses on development and characterization of a novel cell irradiator device which utilizes a pulsed electron-gun to produce low fluence, spatially resolved (~ 1 micron diameter) beams of electrons. The gun will be interfaced with a biological irradiation chamber mounted on an X-Y scanning plate of an optical microscope. Our prototype design involves plating of live cells on a < 1.5 micron thick Mylar membrane which can be aligned over the electron-gun aperture. The second design may involve a masked array with holes spaced several microns apart. Computer simulations of gun performance parameters and mask-interface configurations are underway. Electron tracks used in these simulations were generated and scored using code developed by Professor W. Wilson (Washington State University). The cell morphologies used are derived from confocal

microscopy of Hela-cell monolayers. Breast cells and macrophages will also be examined.

The hypothesis that cells exposed to low doses of electrons and nonexposed neighboring cells respond in a spatially coordinated manner through "bystander" effects will be tested. Studies will be conducted using fluorescent reporter molecules and labeling techniques to determine whether signal transduction pathways involving p53, NFkB and AP-1 are stimulated in both targeted and neighboring cells after low-LET irradiation. Particular emphasis will be placed on elucidating the dose-response relationships for these events and whether modulation of cell cycle progression in bystander cells may influence the shape of the dose-response relationship.

Parallel work on demonstrating and elucidating the fundamental cellular and biochemical mechanisms responsible for the induction of adaptive response will also be carried out. We will establish the relationship between radio-adaptive response and the low-dose-triggered phenomena of Ku protein activation and poly(ADP-ribose) polymerase activation. Whether adaptation is also induced in neighboring, unirradiated cells will be examined by determining if these early steps in adaptive response are induced simultaneously in neighboring, unirradiated cells.

Our collaborative research program will provide: I) a versatile, low-cost irradiation device which will enable cell specific, spatially selective and energy controlled studies, ii) molecular level information on the biological consequences of low-dose, low LET radiation, and iii) quantitative information in the low-dose regime that may be useful in developing accurate long-term risk assessment models for radiation-induced carcinogenesis.

29. The Role of the Number and Spacing of Electron Tracks on the Consequences of Low Dose Irradiation

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Summary: Biological mechanisms which may influence the health risks resulting from very low dose radiation exposures will be investigated using a collimated beam of electrons to simulate the irradiation patterns occurring with low dose exposures.

Abstract: Ionizing radiation produces a variety of free radicals and chemical products which react to produce the same types of oxidative damage in a mammalian cell as produced by the normal metabolic activity of the cell. However, the damage produced by radiation is distributed differently in time and space and may trigger different repair mechanisms or different changes in the growth and differentiation of cells than those triggered by metabolic products. The health risk resulting from a low dose of radiation is currently estimated using the assumption that cells respond individually to radiation induced damage without influence of neighboring cells. Considering how efficiently cells deal with metabolic damage and that cell communication is involved in many growth regulation processes, it seems unlikely that cells would respond independently to irradiation. If they do not react independently, it is likely that the current use of a linear extrapolation to low doses significantly overestimates the risk.

The objective of this project is to evaluate the differences in the response to cells as a function of spatial and temporal distribution of radiation-induced damage. Specifically, we will: (1) Test the hypothesis that normal human

epithelial cells transmit signals to neighboring cells in response to increased levels of oxidative damage produced by low-LET radiation.
(2) Test the hypothesis that the probability of such a signal being received by an adjacent cell is a linear function of the energy deposited in the irradiated cell. (3) Test the hypothesis that the probability of each specific effect in an unirradiated cell is increased in proportion to the number of neighboring cells with elevated oxidative damage levels. And, (4) test the assumption that the signal is passed from cell to cell, but the intensity (probability of causing an effect) decreases in proportion to the distance transmitted. Increased repair related protein expression, apoptosis, and changes in cyclin proteins in neighboring unirradiated cells will be taken as evidence for receipt of a radiation induced signal from a neighboring cell.

Evaluation of the effects of temporal and spatial distribution of damage will be obtained by irradiating selected portions of monolayer cultures with an electron microbeam, delivering individual and counted numbers of electrons simulating beta particles and the secondaries of X- and gamma-ray irradiation to specified portions of the cell culture. The patterns of energy deposition produced by electron tracks in tissues will be simulated, to the extent possible, in the two dimensional tissue culture environment.

This controlled irradiation will make it possible to determine the magnitude of the effect of interaction between cells, and to evaluate the range of the signal which produces this interaction. The apoptotic response of irradiated and unirradiated cells, as well as changes in the expression and localization of repair and cell-cycle control related molecules, will be determined in order to evaluate the validity of linear extrapolations to low doses.

30. Track Structure Simulations for Selected-Cell Irradiation Studies

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Summary: The project will provide computer simulation tools to guide experiments that will expose mono-layer tissue cultures to high energy electrons to investigate potential responses in bystanders.

Abstract: The role of cell-cell communication in tissue responses to ionization radiation is a poorly understood aspect of radiation biology that might give rise to thresholds in the dose responses for important endpoints such as tumor induction. Several experimental systems to study bystander effects by means of selected-cell irradiation are in place or planned. These include positive-ion microbeams as well as broad-beam electron irradiators. By variations in beam intensity and energy, these devices modulate the number and stopping power of particles slowing down or stopping in a selected population of cells.

Computer simulations can contribute to the design and interpretation of these experiments by predicting the distribution of dose at various LETs delivered to subcellular compartments. For electron irradiators, they serve the additional need of estimating dose leakage to masked cells due to multiple large-angle scattering and range straggling.

31. Direct Characterization of the Differences between DNA Damage from Ionizing Radiation and Endogenous Oxidative Processes

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Summary: The aim of our project is to utilize powerful new analytical tools based around capillary separations combined with ultra-sensitive and high resolution Fourier transform ion cyclotron resonance mass spectrometry to identify and quantify the differences in DNA damage arising from ionizing radiation, as a function of radiation dose, in comparison to endogenous oxidative damage.

Abstract: Currently, the health risks associated with exposure to low levels of ionizing radiation are poorly understood, in part because of the lack of appropriate analytical tools for characterizing the distinctive damage products that may be produced. Although the 104 to 105 lesions/cell/day that result from endogenous oxidative reactions are repaired through normal processes, the small fraction of unique or disproportionally produced and unrepaired damage types resulting from exposure to ionizing radiation may lead to deleterious effects. It is important therefore to understand the real differences between DNA damage induced by normal oxidative processes versus low doses of ionizing radiation. Recent advances in analytical methods now provide enormously more sensitive capabilities for efficiently and quantitatively detecting and characterizing the distinctive and chemically complex damage products from ionizing radiation. To this end, we will apply powerful new capillary electrophoresis-Fourier transform ion cyclotron resonance mass spectrometry (CE-FTICR) instrumentation developed at PNNL to quantitatively determine the unique types of DNA damage resulting from ionizing radiation the damage types uniquely resistant to DNA repair. We will use a novel "differential display" approach using distinctive mass tags (e.g., stable-isotope labeling) to efficiently identify and quantitate the unique damage components arising from ionizing radiation compared to endogenous oxidative damage as a function of radiation dose. We will exploit the ultra-high sensitivity and resolution of CE-FTICR and its multi-dimensional MS/MS measurement capabilities to characterize these DNA damage products. Our approach will involve the characterization of damage in oligonucleotides, allowing distinctive and complex damage types (double strand breaks, clustered lesions, etc.) to be effectively determined and that have not been amenable to previous approaches (e.g., involving digestion of DNA to the mono-nucleotide level). In order to understand the differences in DNA damage due to DNA packaging in cells, the studies will involve both "naked" double-stranded DNA and DNA packaged in nucleosomes (i.e., chromatin). We will also use a repair system and the differential display approach to determine the types of DNA damage that are refractory to repair, and therefore to efficiently identify distinctive radiation induced damage types likely having the greatest potential for adverse health effects.

The results of this research will provide quantitative information on the differences in DNA damage resulting from ionizing radiation compared to endogenous oxidative damage. The sensitivity of the approach will also provide a quantitative basis for predicting the levels of ionizing radiation that will lead to production of these unique DNA damage types, and the levels that exceed normal endogenous oxidative damage. Finally, the approach will efficiently identify the distinctive damage types that are less efficiently repaired in cells, and provide a foundation for accurately predicting the threshold for concomitant health effects in humans.

Genetic Susceptibility

32. DNA Repair Gene Variants: Understanding Mechanisms of Cellular Response and Estimating Individual Health Risk from Low-Dose Radiation Exposure

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Summary: Understanding the impact of genetic variation in genes required for repair of damaged DNA on individual susceptibility to cancer is a critical component of the knowledge required to predict the human health consequences of low-level exposure to ionizing radiation.

Abstract: Estimation of health risks from exposures to low levels of toxic agents, including ionizing radiation, is hampered by the large amount of unexplained variation among individuals. Accumulating data suggest that the health consequences of exposure reflect the interaction of dose and the genetic constitution of the individual. DNA repair genes are critical in protecting cells from the consequences of radiation, and repair gene variants with impaired function are known to increase cancer susceptibility. This program initiates a coordinated effort to understand the impact of genetic variation in genes required for DNA repair processes and the maintenance of genome integrity on the health consequences of low-level exposure to ionizing radiation. The focus on identifying variation in genes of relevant DNA repair pathways builds from the observation that reduced repair capacity is a cancer risk factor and the knowledge that specific pathways are responsible for repairing the DNA damage induced by ionizing radiation. The studies address the hypothesis that genetic variants existing at polymorphic frequency in humans in genes relevant to the repair of ionizing radiation induced DNA damage constitute risk factors for cancer incidence in radiation exposed populations. This program focuses on (Aim 1) identification of genetic variation in genes of the Base Excision Repair and the Double Strand Break/Recombination Repair pathways and (Aim 2) developing DNA repair capacity assays as an intervening phenotype to identify individuals with impaired DNA repair function. Molecular epidemiology collaborations will evaluate the role of variants in repair genes in individual susceptibility to disease.

Eight genes involved in the repair of ionizing radiation induced DNA damage have been screened for variation by resequencing DNA of 36-72 unrelated individuals. Eighteen amino acid substitutions have been identified; the average allele frequency is ~0.10. Thus, polymorphic variants in these genes are common. Other variants were identified in searches of public sequence databases. For example, 12 amino acid substitution variants have been identified in Ape1, the major mammalian repair enzyme for baseless sites in DNA. Strikingly, six of the nine substitutions within the repair domain of Ape1 were found to impart reduced repair function (i.e. reduced DNA binding or enzymatic activity in vitro). The studies represent an essential step

towards identifying functionally-significant variation in the human population and in defining the genetic basis for the commonly observed variation in DNA repair capacity and cancer susceptibility within the human population. The variants are reagents for molecular epidemiology studies and several collaborators are finding associations of variants with increased cancer risk or intervening phenotypes presumed to be indicative of cancer risk.

This program initiates a coordinated effort to utilize variant genes as tools to study the impact of DNA repair on population health. Knowledge of the extent of genetic variation in these genes, and especially the variants with potential for conferring differences in cancer susceptibility will provide the basis for analysis of the relative contributions of variation in susceptibility genes and environmental exposure to cancer risk. This is critical data for science-based risk estimation.

33. Sensitivity to Radiation-Induced Cancer in Hemachromatosis

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Summary: To determine if individuals with a defect in their control of iron metabolism (i.e. carriers of the defective gene giving rise to hereditary hemochromatosis) are at increased risk from radiation.

Abstract: Determination of dose-response relationships for radiation-induced cancer in segments of the population with high susceptibility is critical for understanding the risks of low dose and low dose rates to humans. Clean-up levels for radionuclides will depend upon the fraction of the population represented by these people and the lower bounds on their sensitivity to radiation. Most research on susceptibility to radiation has focused on variations in DNA repair genes. However, important segments of the population with conditions that provide a differential growth advantage for mutated cells have not been examined. The long-term objective of this project is to indicate whether people with hereditary hemochromatosis (HH) would display an increased susceptibility to cancer induced by low LET radiation. We propose to pursue this question experimentally with mice having the same mutation as that found in the human disease, HFE knockout mice (Zhou et al., 1998). It is postulated that the major impact of HH will produce a “promotional” environment for radiation-induced cancer. In part this may be attributable to an increased likelihood of developing non-insulin-dependent diabetes mellitus (NIDDM). Development of NIDDM can be influenced by iron overload, but appears to be also found in heterozygotes where iron load is not an obvious factor (Nelson et al., 1995). If the HFE-knockouts are found to have higher sensitivity to radiation than their wild-type counterparts, steep dose response curves are anticipated that can serve to more clearly identify non-linearity or thresholds in the dose-response curve for low LET radiation. A pilot study using HFE-knockout homozygotes and heterozygotes has been designed to:
1) determine whether the knock-out mice have greater sensitivity to radiation-induced cancer of the colon, liver, and breast, 2) establish the dependence of this sensitivity on accumulation of iron, 3) determine the extent to which cell replication and apoptosis occur in these target tissues with varying iron load, and 4) correlate the increases in sensitivity with changes in insulin-related signaling in tumors and normal tissue from each target organ.

34. Genetic Factors Affecting Susceptibility to Low-Dose Radiation

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Summary: The goal of our application is to improve the scientific basis for understanding potential risks to the population from low dose radiation exposure based on potential genetic differences that may modulate an individuals sensitivity to low doses of radiation.

Abstract: The goal of this application is to improve the scientific basis for understanding potential risks to the population from low dose radiation exposure. We propose to address specific genetic factors that affect individual susceptibility to low dose radiation and ask the question do genetic differences exist that make some individuals more sensitive to radiation-induced damage? Is so, could these genetic differences result in sensitive individuals, or sub-populations, that are at increased risk for radiation-induced cancer?

Currently there are two known radiation sensitive sub-populations, which are represented by the diseases Ataxia-telangiectasia (AT) and Nijmegen Breakage Syndrome (NBS). The genes for AT and NBS have been cloned and their protein products have been identified. Both are central players in the cellular response to ionizing radiation. AT and NBS patients are rare and the central premise of this application is that if there really are genetically predisposed, radiation sensitive individuals in the population, then heterozygous individuals, i.e., those individuals that possess only one functional allele for the AT or NBS disease genes are likely candidates.

We have identified the gene and protein deficiency in NBS. In addition we have developed mice that are homozygous mutants for the gene mutated in NBS (NBS1), heterozygous at this loci, and we have the wild-type control mice. We will utilize these unique hypomorphic mice to determine whether there are genetic factors that affect sensitivity to low dose radiation using three specific aims.

Specific aim 1 is to develop, optimize, and validate, a rapid and reliable assay(s) to identify heterozygous animals in our unique mice strain. Our goal is to develop a functional assay by which heterozygotes can be distinguished from DNA polymorphisms in critical genes like those responsible for NBS. Specific aim 2 is to test the hypothesis that our heterozygous mice really are a radiation sensitive population compared with their wildtype littermates when exposed to low doses (1 or 10cGy) of X-rays, either in utero, or eight weeks after birth. If inbred NBS heterozygous are not radiation sensitive then it is unlikely that there really are genetically susceptible radiation sensitive populations. If they are radiation sensitive then a functional assay for heterozygosity is critical.

Specific aim 3 will use those same animals irradiated in specific aim 2 to test the hypothesis that sampling peripheral blood as a function of time after whole body irradiation and monitoring potential radiation-induced genomic instability can be used to predict cancer risk. The biological significance of radiation-induced genomic instability is unknown, and we will directly evaluate whether irradiation induces instability that can be measured in peripheral blood from

exposed animals, and whether this occurs in those animals that eventually develop cancer.

35. Radiation Sensitivity and Cancer Susceptibility

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Summary: The goal of this study is to identify genetic factors that affect individual susceptibility to low dose radiation.

Abstract: Our working hypothesis is that individual variations in radiosensitivity are inherited traits that define risks for radiation-induced cancer. Our approach is to identify radiosensitive and cancer susceptible individuals from an exposed population, then characterize susceptibility factors and identify the responsible genetic elements. Our study population is a group of more than 7,000 individuals who were exposed to total body irradiation (TBI) as part of hematopoietic stem cell transplantation (HSCT) for a hematologic malignancy, non-hematologic malignancy, or a non-malignant hematologic disorder. These individuals have a significantly increased risk of developing new solid cancers later in life as compared to transplant patients who do not receive TBI. The risks of tumor induction are dose-dependent and the tumors often develop at anatomical sites where the radiation dose is highest. Thus they likely represent radiation-induced tumors. We have also observed higher risks of second cancers in patients who developed pulmonary toxicity following HSCT. The development of normal tissue damage such as pulmonary toxicity following radiation exposure often reflects an inherent radiation sensitivity in the exposed individual. Therefore, the higher risks of cancer development in patients who show pulmonary toxicity following TBI suggests a link between radiation sensitivity and cancer susceptibility. We propose to test this hypothesis and identify the genetic elements that underlie radiation sensitivity and susceptibility to radiation-induced cancer. Our study population represents a unique resource with which to address questions concerning the relationship between radiation sensitivity and cancer susceptibility. In addition to the clinical histories of the HSCT patients, we have normal lymphocytes collected prior to any radiation exposure for many of these individuals, and biopsies of the radiation-induced tumors. We propose to continue to expand this data base and tissue bank, and add skin biopsies sampled at regular intervals post-HSCT for longitudinal in vitro studies. These studies will contribute to our understanding of the genetic contribution to radiation sensitivity and cancer susceptibility, and this information will in turn lead to more accurate estimates of individual and population risks from low dose radiation exposures.

Modeling of Risk

36. Stochastic Models for Low-LET Radiation Risk Estimation at Low Dose and Dose-Rate

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Summary: The research will focus on developing a quantitative description of radiation-induced carcinogenesis and use it to estimate risks of cancer associated with exposure to low levels of low LET radiation.

Abstract: The issue of whether the linear-no-threshold hypothesis is still tenable for justifying radiation protection standards at low doses and dose rates is currently the most important single issue in the realm of radiation risk assessment. Unfortunately, most epidemiological studies lack the power to provide a definite answer regarding the validity of this hypothesis at levels of radiation of interest to regulatory bodies. It has been suggested that the development of models based on mechanisms may be one way to provide the guidance necessary to base regulatory standards at the low levels of dose and dose rate encountered in the workplace and for the population-at-large.

The study proposed would include two elements. First, we will use the multistage models currently being developed by our group to study how dose-rate and protraction of exposure might modify carcinogenic response. Consideration of recently discovered phenomena, such as genomic instability and bystander effects, may well change our understanding of low dose and dose-rate response. Second, we will apply the models to the analyses of substantial data sets, both epidemiological and experimental. Combining information from epidemiological and experimental data is important since no single study will have sufficient power to evaluate the health effects of exposure to low levels of radiation.

37. Advanced Computational Approaches for Characterizing Stochastic Cellular Responses to Low-Dose, Low-Dose-Rate Exposures

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Project Summary: The goal of this project is to use novel computational methods to integrate relevant dosimetric, molecular (gene damage, repair, misrepair, mutation), cellular (apoptosis, necrotic death, neoplastic transformation) and other information into low-dose risk assessment for lung cancer induction by radiation and genotoxic chemicals.

Background: We previously published a model for radiation induced neoplastic transformation

called NEOTRANS1. Here, the model is simply referred to as NEOTRANS. With NEOTRANS, one evaluates the probability of inducing persistent, problematic instability (PPI) in the genome of cellular targets, based on model related differential equations. Misrepair of subtransformation damage in DNA leads to PPI among survivors that is passed to cell progeny. Cell progeny with PPI are then modeled as being transformed with a probability that depends on follow-up time. Based on our earlier work, it was concluded that NEOTRANS might not apply to low-LET radiation. While NEOTRANS includes DNA damage, DNA repair, and DNA misrepair pathways, it excludes the apoptotic and necrotic cell death pathways. In addition, macroscopic dose (absorbed dose) is used as the independent variable, rather than the microscopic dose to the critical target. Our current efforts relate to development of a generic model that includes apoptosis and necrotic cell death. We are also investigating the impact of variability in the microdose on the risk for neoplastic transformation. "Biological microdosimetry" was introduced to facilitate this effort.

Specific Aims: The 11 specific aims of this project are too lengthy to report here. However, all relate to developing mechanism-based models for lung cancer induction by low doses of radiation and genotoxic chemicals.

Research Approach: Different approaches are being employed for radiation (*in vitro*) and chemical (*in vivo*) microdosimetry. For radiation microdosimetry, we are currently using a novel biological microdosimetry approach where variability in biological response is used to derive variability in dose to the critical biological target. Bayesian inference is used to generate the microdose distribution. Our approach to biological microdosimetry for radiation does not require detailed information about the mass, volume, or shape of the critical biological target.

Our approach to chemical microdosimetry is partly based on experimental studies carried out at our Institute and is focused on the respiratory tract and on genotoxic, polycyclic aromatic hydrocarbons. Some of the critical parameters used in dosimetry modeling are the molecular diffusivity of a genotoxic agent in the membrane and aqueous portions of living tissues, its lipid/aqueous partition coefficient, and the morphometry and blood flow of the airway mucosa.

Our approach to integrating microdosimetric, molecular, cellular and other information into low-dose risk assessment for lung cancer induction by radiation and genotoxic chemicals is to use Markov chain Monte Carlo (MCMC) methods. The MCMC methods are also used in our biological microdosimetry.

Anticipated Scientific Deliverables:
(1) Mechanisms-based, scientifically-valid risk models for stochastic effects in cells of low doses of ionizing radiation, genotoxic chemicals, or both; (2) microdose distributions to key biological targets relevant to cancer induction in humans by low doses of radiation or a specific genotoxic chemical; (3) validation/invalidation of the linear, no-threshold cancer risk model; (4) peer-reviewed publications of key results; (5) presentations at scientific meetings of key results; and (6) a web site presenting key results with links to other related sites.

38. Models of Radiation Induced Translocations Applied to Human Leukemia Risk Estimation

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Summary: Our goal is to incorporate biological data and biologically based models into the epidemiological cancer risk models for improved low-dose cancer risk estimation.

Background: Biologically-based models offer a means of transferring information from basic science to low-dose cancer risk estimates. This is particularly important for low LET (linear energy transfer) radiation where, contrary to the assumptions used in regulatory policy, there is some epidemiological evidence for low-dose non-linear cancer induction in humans. Human studies are, however, handicapped by their inability to measure risks at environmental exposure levels with any real confidence. It is therefore necessary to bring biologically-based models into the risk assessment process. We will develop mathematical models and other computer simulations of processes relevant to the dose-response of radiation induced leukemias.

Approach: We begin this approach by using the relationship between chromosomal translocations and specifically related leukemia types. The association between radiation and the induction of double stranded chromosomal breaks has been well studied. We first use the BCR and ABL translocation (Philadelphia chromosome) since most all cases of chronic myelogenous leukemia (CML) are associated with this translocation. We have developed a biologically-based linear-quadratic-exponential incidence rate model for radiation-induced CML. The model consists of a linear-quadratic dose-response for the induction of BCR-ABL, a waiting time distribution between BCR-ABL formation and detection of CML, and an exponential cell killing term that multiplies both the background and induced incidence rates. Using data exclusive of the A-bomb survivor cohort, Bayesian priors are defined for each of the parameters in this LQE model. The priors are based on chromosomal translocations in lymphocytes, hematopoietic stem cell survival experiments, CML waiting times in women irradiated for benign disease, the background CML incidence rate in the U.S. population, and genomic DNA target sizes of BCR and ABL.

Scientific Deliverables: The incorporation of translocation data into the epidemiological risk models makes a significant quantitative impact. First the estimated risk of CML per Gy exposure was reduced by a factor of 3 by incorporating the biological data as compared to using only the epidemiological data. Further, the uncertainty in the estimates was reduced so that the upper bound of risk as used in standard setting was only one-fourth that found by using only the epidemiological data. There is next the need to evaluate and quantitate model and data uncertainty.

Clearly by incorporating more biology into the epidemiological risk estimation process makes an important difference in estimated CML risk. It is important to incorporate as much biological information as possible into cancer risk estimates. Plausibility constraints on model structures and parameter values are one means of accomplishing this objective. As we develop further detail and consider other cancer sites the DOE should have a much better quantitative understanding of potential cancer effects from low-dose radiation.

39. Cytogenetic Tests of Radiobiological Models Relating Epidemiologically Measurable Risks to Low-Dose Risks

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Summary: The project will use experiments and mechanistic modeling for chromosome aberrations, which are closely related to cancer but are more amenable to laboratory investigations, to find credible dose-response relations for sparsely ionizing radiation in the range from the low doses mainly relevant to risk estimation to somewhat higher, epidemiologically tractable doses.

Abstract: A priority for risk analysis is understanding dose-response relationships, both in the low-dose range and in an intermediate-dose range where quantitative epidemiology is feasible. The relevant initial damage, such as DNA double-strand breaks (DSBs), is almost surely produced linearly with dose. So the main issues relate to cellular processing of initial damage, which can result in more complex endpoints such as chromosome aberrations and, ultimately, cancer.

Exchange-type chromosome aberrations, produced during the G0/G1 phase of the cell cycle and usually scored at the next metaphase, result from the interplay between DNA repair and misrepair and are highly relevant to carcinogenesis risk estimation. The general goal of our project is using experiments and quantitative modeling to find credible dose-response relations for such aberrations in the low-LET dose range from <0.1 to 2 Gy, i.e. from the low doses mainly relevant to risk estimation to somewhat higher, epidemiologically tractable doses. We will gather experimental information at the higher doses, use computer models to extrapolate to the lower doses, and check the extrapolation with low-dose experiments. We have assembled a team of modelers and radiation cytogeneticists to carry out the following specific aims:

1. Measuring exchange-type chromosome aberrations with several fluorescent in situ hybridization (FISH) techniques after gamma-ray irradiation of human lymphocytes in vitro. Doses used will range from 0.08 Gy up to 2 Gy. Repeated fractions of 0.08 Gy each will also be used, to improve the signal to background ratio. Aberrations measured at 2 Gy will include translocations (closely associated with leukemias), inversions (which have been somewhat neglected in radiation risk estimation but are very probably relevant to carcinogenesis), rings, dicentrics, and complex aberrations (informative about damage mechanisms).

2. Analyzing low-LET aberration frequencies quantitatively and mechanistically, using standard current chromosome aberration models, implemented by extensions of sophisticated CAS (chromosome aberration simulator) Monte Carlo computer software previously written and applied by members of our team. The computational algorithms closely interrelate various kinds of aberrations, simple or complex, and interrelate different doses. In addition to our data, we will model related data from the literature; low-dose results obtained on dicentrics by a large-scale, multi-year, multi-laboratory effort; various recent FISH experiments, usually at higher doses but related to low-dose results by the mechanistic biological modeling; and molecular-level data on mutations at specific loci, also involving higher doses.

3. Determining low-LET dose-response relations for exchange-type aberrations at low doses. In particular, checking if the recombinational-repair ("one hit") chromosome aberration formation pathway, a linear no-threshold molecular mechanism, makes a quantitatively significant contribution to aberration formation at low doses.

The project will result in state-of-the-art computational techniques and mechanistic models for determining risk, which can relate information from cellular and molecular studies at low doses to available data from epidemiological studies. A secondary result will be additional aberration data at low doses. Biophysical and mathematical analysis of aberrations is emphasized as a practical, relatively inexpensive way to help firm-up risk estimates and make them more credible.

40. Biological Effects of Low-Dose and Low-Dose-Rate Radiation Exposures: An Integrated Theoretical and Experimental Approach

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Summary: The goal of this program is to develop a computational method, based on experimental data, which can be used to assess the risks to humans due to low level radiation; this model will allow us to determine the extent to which cleanup is necessary for DOE owned radioactive waste sites.

Abstract: It is widely recognized that to protect the general population from exposure to low levels of ionizing radiation, we must continue to improve our knowledge of radiation-induced cancer risks in humans. It is clear that overall estimates of such cannot be based simply on empirical linear fits of available epidemiological data from relatively high dose exposures - even when adjustments are made for low dose and low dose rate exposures. Such an extrapolation can either over- or underestimate the risks. It is extremely difficult to measure directly small changes in most biological end points, particularly carcinogenesis. Thus, scientifically defensible tools and approaches for determining risk must be developed that can be accepted with confidence. We hypothesize that experimentally determined molecular mechanisms operating at relatively high doses will also be applicable at low doses. Hence, we can develop theoretical models for estimating risk at low doses and low dose rates. Our strategy is to extrapolate mechanisms and not risk from high dose to low dose, taking into consideration those effects that are non-linear with dose.

Specific Aim 1. We will develop geometric models for prototypical interphase diploid mammalian cell nuclei. As data become becomes available, we will map genomic sequence information onto these architectural models of chromosomes.

Specific Aim 2. We will combine our Monte Carlo track structure code for different energy electron tracks with interphase nuclear models to determine the spatial and temporal (for dose rate considerations) distributions of strand breaks and

base damages. Time dependent repair processes will be incorporated into the model.

Specific Aim 3. The misrepair of local clusters of damage will be correlated with point mutations and small deletions. Misrepair of double strand breaks will allow us to evaluate intermediate and large scale rearrangements such as chromosome aberrations. Integration of these mechanisms will allow us to develop a comprehensive, gene specific theory of mutation induction valid at all doses and dose rates.

In order to achieve these specific aims, we will develop comprehensive polymer models of whole chromosomes, incorporating information on the chromatin loops between matrix attachment regions in both heterochromatin and euchromatin. Ultimately, whole nuclei will be modeled including locations on chromosomes of specific genes and regulatory elements important in the carcinogenic process. The development of a general theory of mutation induction will provide the essential foundations for a mechanistically based theory of radiation risk which will be valid at all doses and dose rates. As a starting point, we will incorporate our mutation model into existing multi-stage models of carcinogenic risk, such as, for example, the two-stage stochastic mutation model of Moolgavkar, which includes two mutations (initiation and progression) combined with clonal expansion (promotion). Development of such models will provide the only realistic hope of determining reliably whether there exist threshold effects in the radiation induction of cancer. This will allow rational decisions when evaluating when and how much radioactive waste cleanup is required.

41. DNA Damage vs. Cell Killing by Low-Dose-Rate Gamma Radiation Ultrasensitive Measures, and Implications for Mechanistically Modeled Cancer Risk

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Summary: To obtain information critical to improved radiation-risk extrapolation, this project will develop and apply new, ultra-efficient methods allowing direct comparison of low-dose dose-response (LDDR) relations for DNA damage, mutations, and cell killing in different types of human cells, exposed in vitro to low-level gamma radiation.

Abstract: To obtain information critical to improved radiation-risk extrapolation, we will develop and apply new, ultra-efficient methods allowing direct comparison of low-dose dose-response (LDDR) relations for DNA damage, mutations, and cell killing in different types of human cells, exposed in vitro to low-level gamma radiation. Using these methods, we will examine potential LDDR nonlinearities in cell killing (i.e., “hypersensitivity”) and in DNA damage/mutation (e.g., due to induced DNA repair). We will also explore how induced DNA damage, mutations and cell killing are each affected by cell-division rate during low-level exposure.

Our methods will include parallel applications of:
(1) a new assay for thymine-glycol DNA damage, shown recently to reach zeptomolar (10^{-21} M) levels of sensitivity; and
(2) unprecedented simultaneous/rapid detection of clonogenic death vs. somatic mutations in ~1 million microcolonies/assay, by means of a

novel application of current "gel-microdrop" (GMD) and flow-cytometry (FC) technology. Data involving assay #1 will be obtained in collaboration with ongoing work at the National Cancer Institute of Canada (NCIC). In collaboration with LLNL investigators, data obtained using assay #1 will be supplemented by ultrahigh-sensitivity measures of reduced clonogenic survival based on GMD-FC analysis of the same gamma-exposed cells. To develop/demonstrate assay #2, LLNL investigators will (in Year 1) first modify a line of mutagen-sensitive Chinese hamster ovary (CHO) cells (previously developed at LLNL) to express a FC-detectable fluorescent protein, and then apply existing GMD-encapsulation technology and FC to these CHO cells after low-level mutagenic exposures to demonstrate rapid, ultrasensitive dual detection of reduced clonogenic survival and gene inactivation. Similarly modified lines of human cells will be created at LLNL in Years 2 and 3, to which assays #1 and #2 will be applied in parallel after low-level gamma exposure.

By providing direct comparisons of thymine-glycol damage, mutations, and reduced clonogenic survival in human cells caused by very low-levels of continuous gamma-ray exposure, these studies will reveal LDDR relations critical to improving biologically based predictions of cancer risk posed by low-level exposures to ionizing radiation. Specifically, data obtained will be incorporated into a biologically based cancer-risk model that accounts for competing effects of gamma-induced production vs. killing of premalignant cells. Model predictions will explore how these competing endpoints are likely to shape LDDR for gamma-induced cancer risk. [This work will be performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under contract W-7405-ENG-48.]

Risk and Risk Communication

42. Understanding the Issues in Communicating about Low Dose Radiation Exposure

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Summary: The goal of this research is to understand public perceptions and concerns regarding low dose radiation exposures and to design communications techniques that will support improved public decisions for regulating and managing such exposures.

Abstract: The optimal use of research on the biological effects of low dose radiation will depend upon how well this research is understood by policy makers and utilized to improve the management of low dose exposures. One objective of our research is to improve public acceptance and support of policy and program changes based on the biological research results. At the present time, widespread adverse views about radiation exposure makes communicating with citizens, groups, and organizations difficult. Public concerns about and resistance to existing and proposed strategies for managing radiation exposure are shaped by complex social, cultural, economic, and political factors. We will look for ways to address the existing public suspicion, concern, and opposition.

This research effort proposes a conceptual framework to address the social complexity and the historical development of aversion to radiation exposure. This framework is then used as a guide to identify key points for the study of communication about radiation exposure, to coordinate and integrate the research team efforts, and to structure the coordination with the low dose radiation research program and with other interested parties.

The conceptual framework is defined as the "social geography of risk communication." This framework characterization deals with societal risk responses and the resulting communication by defining five points of interaction: individuals, groups, communities, states/regions, and the nation/society. In many cases these levels of communication and evaluation operate simultaneously and interactively but according to markedly different values, processes, and strategies. The mapping of this "social geography" will focus on forces and conditions that initiate changes in attitudes, opinions, perspectives, and behaviors.

Five basic research topics have been identified. 1) Examination of attitude formation at each level. 2) Study of the role of media and associated social processes. This will address issues of nuclear stigma, trust and distrust in policy and program managers, the role of public interest advocates, and the influence of cost and benefit distributions. 3) The role of negotiations in societal decision making and low dose radiation exposure. This work will examine the potential for compromise and trade-offs. 4) The impact of science on social evaluations of radiation risks. 5) The capabilities and limitations of risk communication messages at different

levels of social geography. This would include the credibility of various stakeholders as sources of information and as analysts of scientific findings.

The methods for addressing these research topics include reviewing the existing literature on individual, group, and community studies, conducting analogous and pertinent case studies, and performing experimental studies (individual and group), and eventually survey research.

Anticipated scientific deliverables: The goal of the research is to provide a strategic understanding of the social context for risk communication and, based upon that information, to develop specific recommendations for meeting public expectations and providing the information needed at various societal levels to improve decision making about low dose radiation conditions.

43. Assessing Research on the Biological Effects of Low Dose Radiation Exposure to Evaluate Human Health Risks

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Summary: The objective of this project is to improve the scientific basis for determining health risks from low dose and low dose-rate radiation exposures by reviewing the scientific literature, producing reports, and implementing a comprehensive communication program for the DOE Low Dose Radiation Research Program.

Abstract: The human population is constantly exposed to low levels of natural background radiation, primarily from environmental sources, and to higher levels from occupational sources, medical therapy, and other human-mediated events. Most of the accumulated research over more than 50 years has focused on the higher levels to formulate and establish exposure standards for protecting the general public and subpopulations such as affected work forces. Although most future human radiation exposures are projected to be at low levels, research has not yielded the type of information that can be readily used to make health-risk assessments related to such exposures. Newer genomic technologies promise to provide the data needed to understand the risks of low-level radiation. In light of these recent advances in genetics and molecular biology, the available data and information must be intensively and thoroughly reviewed and analyzed. This will allow an authoritative assessment of the current status of research that can contribute to a better understanding of the health effects of low dose and low dose-rate radiation.

This project will bring together the necessary data and information on the biological effects of low-level radiation exposure as well as other relevant subjects. The material then will undergo review by expert panels, which will analyze the data and information and prepare reports addressing the following: (1) assessment of the state of low dose radiation research, including recommendations for future research to fill data gaps and minimize uncertainty and extrapolation, (2) research and technological developments supporting the needs of DOE's low dose research program, and (3) information and strategies needed by regulatory agencies to develop risk policies and set standards for low dose radiation exposure.

After review by DOE, the findings of this project and results from the overall low dose program will be made available to researchers, science

administrators, regulators, public constituency groups, students, educators, and the media through a Web site and printed material. Other communication resources will be added as the low dose program progresses, with special emphasis placed on utilizing new Internet technologies as they become available.

44. Optimizing the Scientific, Regulatory and Societal Impact of the DOE Low Dose Radiation Research Program

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Summary: The overall goal of this project is to provide scientific, technical and organizational support to optimize the impact of the DOE Low Dose Radiation Research Program.

Background: The information generated in the DOE Low Dose Radiation Research Program must not only be scientifically sound, but also needs to be conveyed to the scientific community in a open and timely manner and to the general public in a meaningful way. The objective of this project is to help insure that the best science is funded, that the value of the information generated is high relative to its usefulness in policy making and that the program is credible to the public.

Specific Aims: The specific aims of this project are: 1) to provide scientific and technical support for the DOE research program, 2) to facilitate the interactions between DOE, scientists, regulatory and scientific committees, and other government and regulatory agencies, and 3) to provide a focal point for generation of educational materials and communication of research results between the scientists, decision makers and the public.

Deliverables: To meet these specific aims, this project will review the scientific and technical content of all the funded projects and provide them with input and direction. It will help DOE organize, conduct, and summarize meaningful contractors meetings. It will also facilitate other scientific and public forums. An outcome of the project will be timely publication of scientific developments in the open literature for use by the agencies which have the responsibility to make decisions concerning radiation standards. The project will develop mechanisms to convey the information to the general public, such as websites, and educational material for students. These communications will help insure that appropriate and adequate protection from exposures to low levels of radiation is available to the public. These products will optimize the impact of the research conducted under the DOE Low Dose Radiation Research Program.

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