A History of the United States Department of Energy (DOE) Low Dose Radiation Research Program: 1998-2008

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Review Draft

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Preface

This book is the work of a single individual, with considerable technical help, and reflects the author's view on many different research projects. It is important that those that conducted the research review this book and provide feedback that can be used to improve the manuscript. The author appreciates comments and will carefully consider them as the book is updated and published.

The opinions and interpretation of the published data compiled in this book are from the author and do not reflect those of the U.S. Department of Energy or any of the investigators that conducted and published the research.

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Summary

This book provides an overview of the research progress made by the U.S. Department of Energy Low Dose Radiation Research Program over the 10-year period from 1998-2008. It is a useful literature review and provides background information for anyone interested in conducting research on the effects of low-dose or low dose-rate radiation exposure.

The first three chapters describe the state of the field when the Program began, the need for the Program, the development of the Research Program, and the people that played essential roles in outlining the Programs's scientific direction and securing its funding. Chapter 4 describes the technology and molecular techniques developed and combined in research projects. Such combinations illustrate how these advances in technology and biological techniques made it possible to make measurements in the low-dose region where it had not been previously possible.

The research resulted in a number of unique observations that have led to paradigm changes in radiation biology. These are described in detail in Chapter 5 and include bystander effects, genomic instability, and adaptive protective responses. The discussion also illustrates the importance of genetic background on all these observed responses. The observations evaluated in Chapter 5 are further discussed in Chapter 6 to help provide a biological mechanistic basis for the observations. This mechanistic approach will make the data more useful for understanding the impact and implications associated with low-dose responses. Such understanding is essential if any of this research is to be useful in radiation protection and the formation of radiation standards.

Chapter 7 shows how models have been developed to help understand the observations at all different levels of biological organization. Without useful models the basic biological information cannot be applied to radiation standards. Chapter 8 illustrated that without the communication of the information, not only to scientists but to the larger community, the information is of limited value. It is thus essential to have adequate communication before scientific information can be useful. Chapter 9 looks to the future to determine how such complex and abundant data can be integrated and interpreted to understand and predict radiation risks in the low-dose and dose-rate region. The "systems approach" is a discussion of such integration techniques.

The final chapter is a discussion of impact of the program on standard setting and radiation protection. To date, these data have had major impact on understanding the biological processes triggered by low doses of radiation but require additional research, development of methods of using the data, and communication before such data can impact radiation standards.

Acronyms and Initialisms

ALARA	as low as reasonably achievable
ALS	Advanced Light Source
ANL	Argonne National Laboratory
ATM	ataxia telangiectasia mutated protein
BER	biological and environmental research
BNL	Brookhaven National Laboratory
CHO	Chinese hamster ovary
DDREF	dose and dose-rate effectiveness factors
DIE	death-inducing effects
DSBs	double-strand breaks
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
EPA	Environmental Protection Agency
FISH	fluorescent in situ hybridization
FNA	French National Academy
GCI	Gray Cancer Institute
Gy	Gray
HFC	human fibroblast cells
HMEC	human mammary epithelial cells
HRS	hyper-radiosensitivity
HZE	high Z
ICRHER	International Consortium for research on health effects of radiation
ICRP	International Commission on Radiation Protection
ICRP	International Council on Radiation Protection
IRR	increased radioresistance
ITRI	Inhalation Toxicology Research Institute
LBNL	Lawrence Berkley National Laboratory
LC-MS/MS	liquid chromatography/tandem mass spectrometry
LET	linear energy transfer
LLNL	Lawrence Livermore National Laboratory
LMDS	locally multiply damaged sites
LNT	linear-no-threshold
MnSOD	manganese superoxide dismutase
NAS	National Academy of Sciences
NASA	National Aeronautics and Space Administration
NCRP	National Commission on Radiation Protection
NCRP	National Council on Radiation Protection and Measurements
OMB	Office of Management and Budget
ORNL	Oak Ridge National Laboratory
PCC	premature chromosome condensation
PNNL	Pacific Northwest National Laboratory
RBE	relative biological effectiveness

ROS	reactive oxygen species
SCE	sister chromatid exchanges
TGFβ	transforming growth factor beta
TRDLs	tandem repeated DNA loci

Chapter 1

Introduction

This book documents the first ten years of the U.S. Department of Energy (DOE) Low Dose Radiation Research Program. It provides information about the development of the Program, the scientists involved, and the scientific progress and impact on the development of low dose radiation information that could impact the setting of standards.

An important goal of this book is to summarize the impact of the Program's research on the current thinking and low dose paradigms associated with the radiation biology field. An additional goal is to help stimulate research on the potential adverse and/or protective health effects of low doses of ionizing radiation. The final goal of the book is to provide a summary of the data generated in the Program that will provide a scientific basis for setting radiation standards.

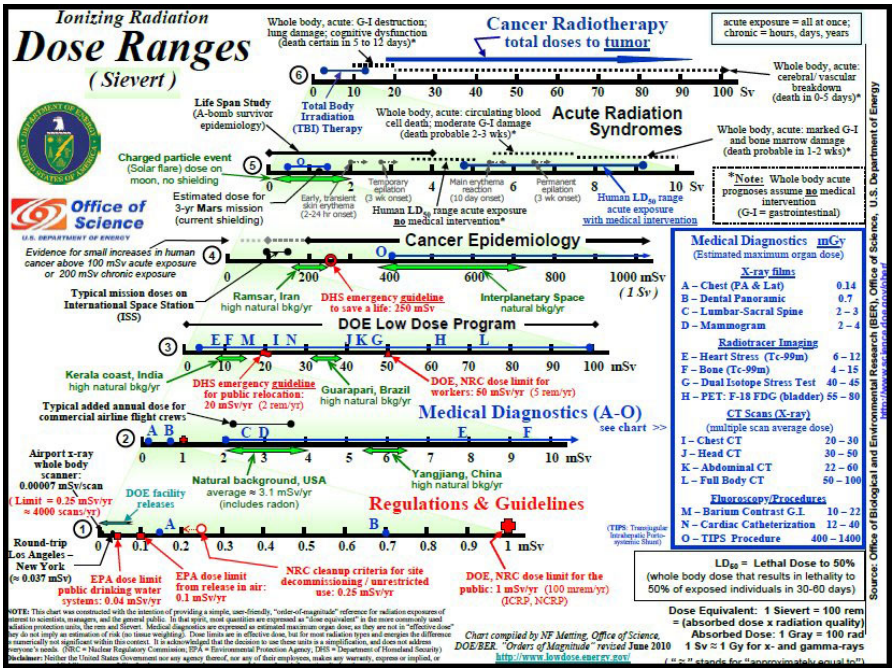
The Program's research has provided extensive information that has helped define biological responses in the low dose region. Serious challenges remain and must be resolved before this information can be used to determine adequate and appropriate standards associated with the risk from low dose radiation exposures.

It is important to note that most researchers involved in the DOE Low Dose Radiation Research Program were conducting similar research with funding from other sources. This book is not a review of all the literature developed by the scientific community on low dose radiation effects, but focuses on the information produced with funding from the DOE Program. The author recognizes that there may be some Program publications that have been missed and not included in the book and apologizes for these inadvertent omissions.

Historically, radiation risks in the low dose region have been difficult to evaluate for two major reasons. The first is background radiation, which is modified by elevation, geographic location, environment, and diet, and is therefore variable over a wide dose range. Background radiation also includes many man-made exposures to radiation in the low dose region from a wide range of sources, including military activities, medicine, and industry. All of this background radiation influences the total dose that individuals and populations receive. Second, a high spontaneous frequency of cancer and genetic effects exists that is influenced by many factors, including lifestyle, genetic background, and environmental conditions. These make detection of any low dose radiation-induced effects very difficult.

The relationship between specific human radiation doses and many biological responses has been reviewed extensively by national and international groups and has resulted in many publications from the National Commission on Radiation Protection (NCRP), the International Commission on Radiation Protection (ICRP), the National Academy of Sciences, and other agencies and regulatory groups associated with radiation protection. Dr. Noelle Metting, program manager of the DOE Low Dose Radiation Research Program since 2000, developed a chart that clearly and

simply summarizes the many orders of magnitude of the radiation exposures that people were and continue to be exposed to and compares the level of radiation allowed in radiation protection standards to other common human exposures. These are presented in Figure 1.



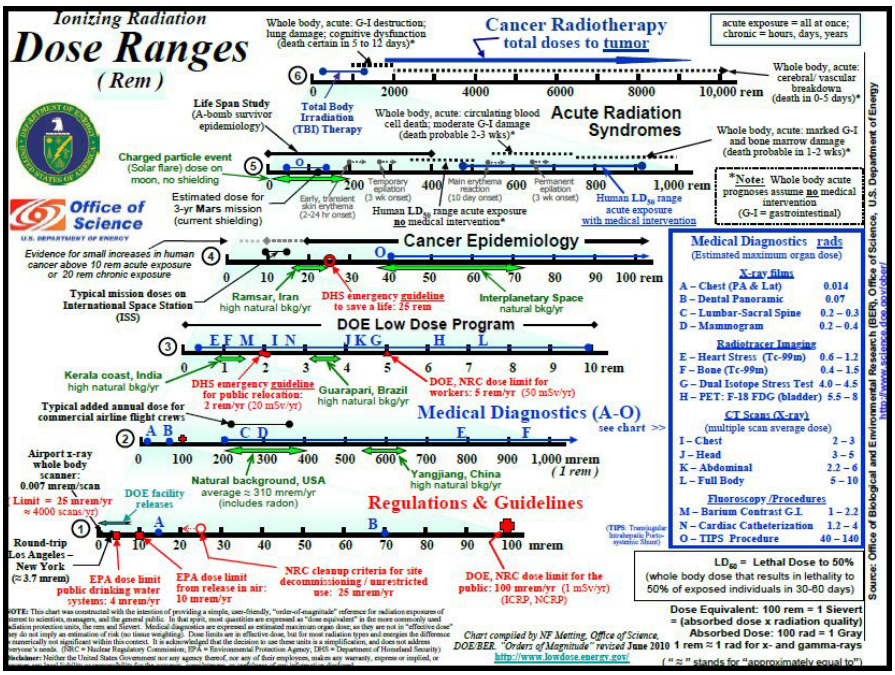


Figure 1. Dose range charts developed by Dr. Noelle Metting. The exposures are given on a log scale, and each line shown represents a dose 10 times greater than the previous line. It shows doses experienced in everyday life relating to current regulations, background radiation, research

doses, acute radiation effects, and radiation therapy. A) international units (Gray and Sievert), B) conventional units (rad and rem).

These figures relate the radiation exposures from many specific human activities to some of the biological effects that are seen at these dose ranges. They also illustrate the doses under investigation in the Program and show that in this dose region, it is not possible to detect changes in the frequency of cancer or genetic effects in human populations.

The inability to detect these changes was one of the driving forces involved in the Program's initiation. It was felt that to ensure that radiation protection standards are adequate and appropriate, there must be a better understanding of the mechanisms of interaction of radiation with biological systems in this low dose range and the possible impact of these exposures on radiation risk. These figures and discussion illustrate the challenge and importance of detecting and understanding the biological changes induced at this very low dose that the Program faces.

Although the radiation responses in this low dose region has not been detected, they have been predicted by models, and there has been extensive discussion and questions associated with the current regulations in this dose region. Radiation protection standards are set (after adjustments for radiation type, dose, and dose rate) using the Linear-No-Threshold (LNT) model. The LNT model constructs a straight line from the observed health effects induced by high doses to the predicted health effects of low radiation exposures.

These estimates are used to predict cancer risks in the low dose range where changes in cancer frequency cannot be detected. There is thus, a degree of confusion between measured data, real risk, and extrapolated risk—risk that cannot be supported by data. This extrapolation was based on old radiation paradigms. Research by the Program has generated data that impact many of these paradigms. These data are reviewed and summarized here to provide a scientific basis for further evaluation and evolution of the current thinking in the fields of radiation biology and radiation protection.

When low dose research was initiated, several well-accepted radiation paradigms existed. The Program's research has demonstrated that some of these paradigms may need to be challenged. For example, it was assumed that the cell was the important biological unit for determining radiation response, and that energy deposited in an individual cell was responsible for the biological effect observed in that cell. This was called the "hit" theory. Results from recent research using microbeams and other techniques developed by the Program have demonstrated that this is not the case, and that "bystander" cells, which have no energy deposited in them, also can respond to radiation exposure with a wide variety of different changes. Some of these biological changes seem to be damaging, and others are protective.

Another widely held paradigm was that cells act independently of each other and that cancer risk could be evaluated based on single cell responses. It has now been well established that there are extensive cell-cell, cell/matrix, and tissue/tissue interactions that determine the outcome of any radiation exposure and demonstrate that it takes a tissue to produce a tumor. Cells respond very

differently in complex tissues than they do in tissue culture, and these complex responses have been evaluated by the Program and must be considered in risk estimates.

Another paradigm was that a single cell "hit" by radiation could produce a single rare event, such as a mutation, that was inherited by the daughter cells and was the most important change during the induction of radiation-induced cancer. Research has demonstrated that in addition to causing mutations, 1) ionizing radiation changes the gene expression in many genes; 2) gene expression is altered as a function of radiation dose, with unique low dose and high dose genes identified; and 3) changes in gene expression can change the fate of the cells in terms of many biological endpoints. This suggests that very different mechanisms of action are involved following exposure to low doses of radiation than those activated by high doses. This further suggests that it may not be possible to extrapolate radiation effects linearly from high doses to low doses, as is currently done.

A final paradigm was that a mutated cell passed on its mutation to each cell in subsequent generations and that this was the basis for the induction of cancer from radiation. It has been demonstrated that, in addition to mutations in individual cells, radiation can also produce genomic instability in cell populations, and this instability can be seen only after many cell divisions. The role of genomic instability in radiation-induced cancer, especially at low doses, is a major paradigm change and a major focus of research in the Program.

It is important to know if the dose-response relationships are linear in the low-dose region or if the response is less or more than that predicted by a linear dose-response curve in the low dose region. Thus, the shape of the dose-response relationshipsfollowing low doses remains one of the most important questions in the field of radiation biology. Before the Program was started, it was demonstrated that small radiation doses could decrease the response of cells to the induction of chromosome aberrations and mutations produced by a subsequent high dose of radiation. This was called the "adaptive" response. Research conducted by the Program has demonstrated that adaptive responses can occur in many animal, tissue, cell, and molecular systems at low doses and not only modify the response to subsequent high doses but also decrease the background frequency of the endpoints of interest. This may suggest the possibility of a radiation-induced decrease in cancer risk in the low dose region. The mechanisms involved in these and other responses are being carefully researched and are reviewed in this book.

As new research in the Program was conducted, it became obvious that new radiation paradigms are needed to describe the response of biological systems to low doses of radiation. This research has moved beyond simply descriptions of the new phenomena of "bystander effects," "adaptive responses," "changes in gene and protein expression," and "genomic instability. It has developed the basis for a mechanistic understanding of the interaction of radiation with complex biological systems. With such mechanistic understanding, it will be possible to develop standards based on more of a systems approach that considers all the biology involved in radiation-induced changes. It is hoped that this research will provide a solid scientific basis for adequate and appropriate radiation standards.

Chapter 2

A Brief History of Radiation Biology

I. The A-Bomb

From the time that radiation was discovered until the development of the atomic bomb (A-bomb) in the 1940s and on to the present, there have been concerns about and research conducted on the health effects of radiation. Animal models provided the main source of information to test the early biological effects. By the time the first A-bombs were dropped on Hiroshima and Nagasaki in 1945, scientists had a good idea of what many of the acute effects of radiation exposure were. The acute radiation syndrome induced from high acute doses of radiation delivered to the A-bomb victims followed a similar pattern to that observed in animal models. Extensive research continued on the acute radiation syndrome, and it has been very well characterized. However, at the time of the A-bomb there was limited information on the long-term effects of radiation, especially as the radiation dose decreased below about 0.5 Gray (Gy). There was also very limited information on the deposition, distribution, dose, or health effects from the radioactive materials present in fallout from the A-bomb.

These concerns resulted in the development of extensive radiation biology programs at DOE national laboratories in the United States (Oak Ridge National Laboratory (ORNL), Brookhaven National Laboratory (BNL), Argonne National Laboratory (ANL), Lawrence Livermore National Laboratory (LLNL), Lawrence Berkley National Laboratory (LBNL), Pacific Northwest National Laboratory (PNNL)), as well as national laboratories in England, Germany, France, and Japan.

In addition to the national laboratories, extensive research was funded at a number of specialty laboratories to look at specific problems such as the inhalation of radioactive materials (e.g., Lovelace Inhalation Toxicology Research Institute). Research on the health effects of radiation also began in several U.S. universities. Major universities with long-term radiation research programs involved in this early research included the University of Utah, Cornell University, Colorado State University, Columbia University, University of Wisconsin-Madison, University of New York at Rochester, University of California at Berkley, Case Western University, Harvard University, University of Tennessee, and the University of Texas. Many other universities had smaller research programs. With the initiation of the DOE Low Dose Research Program, many more universities are currently involved in research on the effects of radiation.

This introduction does not provide details or references to any of this research. It is only included to 1) provide a very brief description of the state of knowledge on the health effects of radiation at the time the Program was started, 2) outline the extensive research that had been conducted on the impact of radiation on living organisms, 3) illustrate the very large database that existed, and 4) set the stage for the need and development of the Program.

II. Early Effects of Acute Radiation Exposure

Early research was focused on the acute effects of radiation. This research defined the radiation damage to organs and tissues that resulted in early deaths from the *acute radiation syndrome*. Studies were conducted on large numbers of different animal species, and it was determined that the amount of radiation required to kill half of the animals in 30 days ($LD_{50/30}$) depended on dose-rate and the species. The studies determined which organs were responsible for the deaths as a function of dose and time after exposure. This resulted in the definition of the prodromal (or initial) *acute radiation syndrome*, which was further evaluated to reflect the tissues affected.

At very high doses, the nervous system was destroyed, and death resulted in a matter of hours and days. As the dose decreased, the cause of death stemmed from the gastrointestinal system. As cells lining the gut died, fluid loss, infections, and death occurred in the first few weeks after radiation exposure. At still lower doses, the cells in the blood system were depleted, resulting in death in one to two months. Deaths from failure of each of these systems were studied very extensively and are well defined.

Several *in vivo* tests were also developed to evaluate cell killing in whole animal systems. These different systems showed many similarities among species as well as some interesting differences that helped understand radiation sensitivity and resistance. The animal studies were related to the information on humans exposed to the A-bomb. Radiation accidents provided additional human data. Many of the animal studies were predictive of the systems' failures induced by the radiation in humans, and thus the type of early damage that resulted in deaths. An interesting observation that came out of the high dose acute effects research was that some animals and people were much more radiation resistant and able to survive these early radiation effects, and others were very sensitive. This was related to their genetic background. The influence of genetic background on radiation-related genetic sensitivity and resistance remains one of the major areas of focus for research in the DOE Program.

III. Late Effects Induced by Radiation Exposure

Concerns about late effects of radiation, especially cancer and genetic effects, led to extensive research in Japan to follow the A-bomb survivors to determine the role of radiation dose on inducing late effects. In addition to the human studies, several carefully controlled animal studies using a range of different species were also conducted where the animals were exposed to graded doses of radiation that did not result in acute lethality. They were then followed for their lifetime to determine induction of both genetic effects and late effects such as cancer and heart problems. These animal studies provided additional support to the human data, helped understand the mechanisms involved in the development of late effects, and studied these endpoints under carefully controlled conditions.

All of these high dose studies, especially the study of the A-bomb survivors, provide the major information used in calculating health risk effects from radiation exposure. These studies have been extensively published, and they are well summarized in reports from the National Academy of Sciences (NAS), National Council on Radiation Protection (NCRP), the French National Academy (FNA), and the International Council on Radiation Protection (ICRP).

Research was also conducted to determine the health effects of fallout from nuclear weapons and reactor accidents. Early studies focused on radiation ecology, the movement of radioactive materials through the environment. These studies determined that different radionuclides were concentrated or discriminated against as they moved through the food chains. Such studies provided the information needed to determine radiation dose and dose-distribution in humans from the many radionuclides in radioactive fallout.

These studies were important because of the extensive atomic weapons testing that took place aboveground from 1945-1962, which resulted in global contamination from fallout. The United States conducted just over 100 aboveground nuclear weapons tests at the Nevada Test Site, and the Former Soviet Union tested close to 300. The rest of the nuclear community also tested nuclear weapons resulting in the spread of radioactive fallout throughout the world. Radiation ecology studies were also necessary to define the movement of the radioactive fallout from the environment to man and for the safe development of nuclear power.

Extensive studies were conducted on health effects from internally deposited radionuclides that resulted from the fallout. This research used a several different animals, with a focus on the beagle dog. The dog studies have been summarized in several books that are discussed in chapter 7 and in multiple proceedings of scientific meetings. Each laboratory that conducted these studies with government funding was required to produce an annual report. These reports provide a large database from the laboratories that conducted this research: PNNL, ANL, BNL, the University of California-Davis, University of Utah, and Lovelace Inhalation Toxicology Research Institute (ITRI). Extensive research on the retention, distribution, dose, and health effects of internally deposited radioactive materials was also conducted in England, France, Japan, and Germany.

IV. Mechanistic Cell and Molecular Studies

A. Cell Killing

Animal studies were supported by cellular and tissue studies that evaluated the mechanisms involved in induction of these early and late effects. These studies were conducted at the national laboratories, specialty laboratories, and universities throughout the world. The ability to grow cells in culture enabled researchers to focus on radiation's role in cell killing. Two basic types of studies were conducted: 1) colony formation assays, or the influence of radiation on the ability of cells to survive, divide, and form colonies; and 2) dye exclusion assays, or the ability of cells to stay alive regardless of their ability to divide. Most of the early studies used colony formation assays.

At this time, the cells used in the culture systems were fibroblasts. By supplementing the media with fetal calf serum, these cells could grow in culture, divide, and form colonies that were easy to measure and provided a direct way to determine the ability of radiation to kill cells, or at least limit their ability to grow and form colonies. However, this cell type was not ideal for understanding cancer, because most cancers arise from epithelial cells. As research progressed media was developed which made it possible to grow epithelial cells in culture. This resulted in

more realistic dose-response relationships for cell killing, chromosome damage and mutation induction.

Many well-characterized immortalized cell lines were developed during this time that could be used by many laboratories. Despite their limitations, cell survival studies were very useful in defining the influence of radiation type (alpha, beta, gamma, and neutron exposure), exposure characteristics (dose-rate, dose fractionation, or Linear Energy Transfer (LET)), and chemical protection on cell survival. The slopes of the dose-response relationships and the shape of the dose-response curves were defined as a function of all these variables. This provided very useful information in understanding the mechanisms involved in radiation-induced cell deaths following large radiation exposures.

In these *in vitro* studies, the sensitivity to detect changes in the very low dose region (<0.1 Gy) was limited, so most of the studies used a dose range of 0.5 Gy up to many Gy, or to the point where most of the cells were killed. The cell-killing curves developed for low-LET radiation showed an apparent plateau in the low dose region with an exponential decrease in cell survival as the dose increased. On the other hand, exposure to high-LET radiation showed an exponential decrease in cell survival over the whole dose range. Many studies were conducted to determine factors such as dose rate, dose-fractionation, and genetic background of the cells that would influence the shape of the dose-response curves.

The DOE Program-funded research played an important role in the development of new techniques to measure cell killing in the low dose region. These new data will be reviewed in Chapters 4 and 6 and the implications for risk discussed in Chapter 10.

B. Mutation Induction

In the early days of radiation biology research, a primary concern about radiation exposure was the potential for the exposure to increase the mutation frequency. Early research on mutations in *Drosophila* and other test organisms suggested that there was a linear dose response for the induction of mutations over a wide range of doses and that there was little repair of this radiation-induced genetic damage. This implied that there was a linear no-threshold increase in genetic damage as a function of radiation dose, and that each unit of radiation would increase genetic risk. The very early data suggested that genetic damage from radiation would accumulate across generations and eventually have a marked impact on the health of human populations. However, extensive research in mammalian systems showed that there was significant repair of radiation-induced genetic damage, and that the damage was dependent on dose rate, sex, and many other factors. Surprisingly, the studies on A-bomb survivors and their offspring did not detect radiation-induced genetic effects.

Research at ORNL used mice (mega-mouse studies) to evaluate the induction of mutations in mammals. They irradiated male mice with large acute doses (3.0 Gy), just below the level to induce lethality, let them recover, and gave a second 3.0-Gy dose. They then mated these mice and evaluated the induction of mutations in the offspring in specific genetic (loci). The frequency of mutations transmitted to the offspring at these loci could be related to the radiation exposure and dose. Many other studies were conducted on female mice, as well as studies on mice exposed to different radiation types, dose-rates, and dose fractionation. Additional genetic

endpoints were used in many of these studies. These studies have provided a valuable data set on which the genetic risks from radiation exposure were based.

Studies were conducted at LANL to determine if the frequency of genetic damage increased after irradiation of many generations of mice. These studies determined there was no buildup of genetic mutations over the many generations exposed and that the reproduction process seemed to limit the transmittal of genetic damage to offspring. They also determined that there were differences in radiation sensitivity between sexes, strains, and dose patterns. The studies also quantitatively measured the repair of genetic damage and helped to explain the lack of buildup of genetic damage following radiation exposure over many generations.

Animal research provided a usable dose-response relationship for the induction of mutations. The database developed in these studies still provides one of the major inputs for estimating the genetic risk in humans. Because of the low risk of induction of genetic disease it was concluded that the radiation risk for genetic damage was small relative to the risk for radiation-induced cancer, and most research focused on radiation-related cancer.

Because of the low frequency of mutations detected per unit of radiation dose, it was not possible to make many measurements in the low dose region. Developments of techniques to measure DNA damage and mutations in the low dose region have since been developed and will be discussed. The implications of radiation-induced DNA damage, mutations, changes in gene expression, and other genetic alterations will be evaluated extensively in this book.

C. Chromosome Aberrations

Another indication that radiation impacts can instigate changes in genetic material was the early observation that radiation causes chromosome breakage and rearrangements. These changes were also found to be present in many types of cancer. It was predicted that the frequency and types of chromosome aberrations provided a good measure of cancer risk following radiation exposure. Techniques were developed to culture human blood lymphocytes and to measure the frequency of chromosome aberrations in these cells when they were exposed in either tissue culture or individuals. The response was the same. Chromosome aberrations thus provided the most sensitive biological change that could be used to detect radiation-induced damage. The frequency of chromosome aberrations was carefully related to radiation dose and became a useful bio-dosimeter. The frequency of chromosome aberrations in blood lymphocytes still remains the gold standard to estimate radiation exposure in human populations exposed in radiation accidents where little or no other type of dosimeter is available.

The literature on radiation-induced chromosome aberrations is extensive and includes measurements made in humans and experimental animal systems. Techniques have been developed that make it possible to stain each chromosome a different color so that the frequency, location in the genome, and type of radiation-induced damage can be carefully measured. Development of molecular and cellular techniques to measure radiation-induced changes in the type and frequency of chromosome aberrations following low doses of radiation in a number of different tissues will be discussed in this book. Such research has resulted in increased efforts to determine the usefulness of chromosome aberrations in estimating radiation dose and predicting human cancer risks from radiation exposure.

D. DNA Damage and Repair

DNA was postulated to be the most important target molecule modified by radiation in the process of radiation-induced cancer and genetic effects, resulting in a very large number of studies focused on the ability of radiation to induce DNA damage. Studies were also designed to explore the mechanisms and repair of that damage. In the early days of research on DNA damage, the endpoints measured were dependent on large amounts of DNA being damaged. This resulted in the early studies being conducted *in vitro* following very high doses of radiation. These basic mechanistic studies determined the types of DNA damage induced by radiation and defined different repair types and pathways. This information still provides one of the best links between radiation-induced DNA damage and the induction of cancer and genetic effects.

It was also suggested that radiation results in a different type of DNA lesion than that produced during normal endogenous oxidative metabolism. Exploring the differences between radiation-induced DNA damage and damage produced by normal oxidative metabolism was initially identified as a major research focus and remains one of the important elements of the Program. Techniques were developed to measure DNA damage in individual cells that made it possible to study the damage following the lower radiation doses relevant to the Program.

Recent developments will be discussed that link the radiation-induced DNA damage to signaling pathways. This modern research has demonstrated that not only are the DNA alterations, breakage, base substitutions, and rearrangements important in cancer but that these radiation-induced DNA alterations trigger many signaling pathways. These pathways regulate the cell's response to radiation, such as cell cycle changes, differentiation pathway changes, and alterations in gene induction and expression. Many of these changes can be detected, even following low doses of radiation. The impact of these unique alterations in signaling pathways triggered by DNA damage remain an important area of research for the Program.

Chapter 3

Need for the DOE Low Dose Radiation Research Program

I. Background

Chapter 2 illustrates the extent of the research on the health effects of radiation from the beginning of the nuclear age. Public concern, particularly after the A-bomb, promoted research that developed an extensive database on the health effects of radiation. At the time the DOE Low Dose Radiation Research Program began, the scientific community knew more about the health effects of ionizing radiation (at high doses) than any other environmental risk factor.

Radiation effects had been measured following exposure to all different types of radiation, delivered over a range of radiation dose rates and by many exposure modes. These high dose acute radiation effects were carefully characterized in numerous molecular, cellular, and experimental animal systems and human populations. Many reviews of these data by national and international committees were used to establish risk estimates. The risks associated with exposure to these higher doses of radiation have been very well characterized, and current risk estimates in the high dose region are supported by the extensive scientific and human exposure data.

The movement of radioactive material in the environment is also well characterized. Radioactive materials are naturally transported through the environment, taken up into living systems, and can eventually result in incorporation of radionuclides into humans. The uptake, deposition, distribution, dose, and biological effects of the non-uniform distribution of radiation from internally deposited radioactive materials are well known in both animal models and humans and are supported with a vast amount of scientific data.

The use of radiation in medicine is widespread, and large human populations are exposed to radiation daily for diagnosis of diseases and cancer therapy. These populations remain an important concern. Follow-up on the long-term effects that may be produced by this exposure will further help establish risk estimates from low doses of ionizing radiation.

Finally, the large number of people exposed to high and low radiation doses following the A-bomb and to local fallout world-wide from nuclear testing have been studied carefully and represent the gold standard for radiation epidemiology studies. Additional human exposures from accidents associated with the development of nuclear energy and in the medical field provided additional important information on health effects or ionizing radiation. The health effects of radiation on these large human populations have been extensively reviewed and evaluated in many epidemiological studies and the risks from the exposure characterized (NRC 2006).

However, despite all the research that has been done, there is a lower limit to the level of energy deposition, exposure, and dose that can be related to radiation-induced disease using human

epidemiology methods. This limit is related to several factors that prevent the detection of radiation-induced cancer and genetic effects in humans. These include:

- Variable levels of background radiation
- High and variable human cancer frequency
- Multiple environmental factors and lifestyles that influence cancer frequency
- Problems associated with determining dose and exposure in human populations
- Difficulty in defining appropriate exposed and control populations
- Cost and scientific efforts required for long-term follow-up of exposed human populations to determine the health effects produced by radiation.

Additional late-effect problems particularly associated with cancer are related to the long time period between radiation exposure and development of cancer. Finally, it is not possible to determine if any cancer observed in either the exposed or control population was induced by the radiation or from other causes.

These problems made it necessary to develop models to predict cancer in the low dose region. There has been extensive debate about these models and the shape and slope of the dose-response relationships because of the inability to determine the risk for health effects in this area. This uncertainty of the models used to estimate risk and the inability to accurately predict cancer risk in human populations exposed to low doses of radiation were recognized by the scientific community and the public and were major scientific factors involved in establishing the DOE Program to fund additional research in the low dose region.

II. Development of the DOE Low Dose Radiation Research Program

The need to determine the radiation risk in the low dose region was recognized at the highest levels of government. This was especially true when discussions of the expenses associated with radiation protection, environmental clean-up, and nuclear waste storage were considered. Senator Peter Domenici, a Republican from New Mexico and Chairman of the Senate Appropriations Subcommittee on Water and Energy for many years, was a strong supporter of basic radiation research and made many presentations where he outlined these expenses and the uncertain nature of the risks in the low dose region. He suggested that the use of the LNT model for estimating these risks resulted in much of the expense. He was one of those in government leadership who recognized the need to have radiation protection standards based on the best possible science.

The DOE Low Dose Radiation Research Program needed and received strong support at the scientific, political, and government agency level. Supporters such as Sen. Domenici and those working in the DOE were essential to get the Program started and provide the funding needed.

In 1990, Dr. Marvin Frazier, a Pacific Northwest National Laboratory scientist who had a very strong background in radiation biology as well as molecular biology, was hired by DOE Headquarters as a Technical Representative for the Office of Biological and Environmental Research (OBER). This position provided scientific input on important political and program decisions. One of his early assignments from BER Director Dr. David Smith was to review the

status of the research being conducted by the DOE in the field of radiation biology and to determine what parts of the research could be justified to continue to receive funding. This justification was to be based on research that would contribute new knowledge that could be applied to setting radiation standards.

At that time, much of the funding for radiation biology research was invested in life-span studies on the health effects of radiation on dogs. These studies had been conducted at the national laboratories, universities, and in specialty laboratories for more than 20 years. DOE's review of these programs found that they provided valuable information on the risk and health effects of internally deposited radioactive materials. Because limited information existed on these effects on humans, the dog study data were essential and were used in setting standards for these effects.

The studies provided understanding of the risk associated with changes in dose rate, dose distribution, LET, physical and chemical properties of the radionuclides, and the route of administration and uptake. They also established the relationships between uptake, distribution, and retention used to calculate radiation dose and dose rate from a wide range of internally deposited radioactive materials. This research created a valuable database that made it possible to relate the dosimetric variables to the biological effect in each individual dog. Thus, it was possible to treat individual dogs as clinical subjects, follow the development of pathology related to radiation in each dog over its lifetime, and relate the biological changes induced in the animals to dosimetric parameters. The dog studies helped establish factors used to set standards such as the influence of non-uniform dose distribution, the risk per unit of dose for different types of radiation exposure (w_D), a wide range of different radionuclides and exposure types, tissue weighting factors (w_t), and the dose and dose-rate effectiveness factors (DDREF).

The DOE assessment of the dog programs suggested that, although these studies had been valuable, continuing the high level of funding would have limited promise for future mechanistic research or impact on standards. The programs were given 2 years to wind down and publish data. For several years after these projects were terminated in the mid-1990s, DOE provided limited radiation biology funding. The funding was decreased because the LNT models were thought to be conservatively protective, and the scientific tools and methods available in epidemiology and toxicology were inadequate to address questions associated with cancer risk following low doses of radiation.

The initiation of the DOE Low Dose Radiation Research Program was the major area of focus for renewed research in the DOE on the health effects of radiation. To understand how the program got funded, it is important to briefly discuss the players and interactions involved in getting political and agency support for the Program.

In my discussions with Dr. Frazier, he emphasized that it was important to understand how developing credibility for BER with the Office of Management and Budget (OMB) was needed to initiate the Program. Dr. Frazier's experience with OMB started in at hearings on Air Quality Standards, for which he reviewed documents. He suggested that the expert review group being used by OMB seemed to be picking data to meet their preconceived ideas. He wrote a paper and presented it at their meeting, which caused the committee to take a recess and discuss his suggestions. As a result of this meeting and interactions with OMB, particularly with Dr. Gary

Venethum, OMB Branch Chief, Dr. Frazier and BER gained the needed credibility from OMB to help get the funding for the DOE Low Dose Radiation Research Program.

In the early 1990s, two of Sen. Domenici's staff, Peter B. Lyons and Alex Flint, began working to determine the need and direction for a new research program in radiation biology to help define the risk in the low dose region. With the information they had derived they came to DOE-BER and worked to help develop the program. Dr. Ari Patrinos, Division Director of BER, was given the charge to draft a scientifically sound program. He and Dr. Frazier wrote a draft description of what the program would be and presented it to OMB, which agreed that the program would be worth doing and had them proceed with its development.

They suggested that a major goal of the new program would be to generate data that would determine if the use of the LNT dose-response models, the use of collective dose, the concept of "as low as reasonably achievable" (ALARA), and the current DDREF effects used in standard setting were supported by and could be tested using the modern genomics approaches and new tools only recently made available.

After much work between the DOE staff, Sen. Domenici's staff, and the OMB, the Program began in 1998. Sen. Domenici's continued support of the program was evident in a quote from a talk at Harvard University October 31, 1997: "In this year's Energy and Water Appropriation Act, we initiated a ten-year program ($13 million/year) to understand how radiation affects genomes and cells so that we can really understand how radiation affects living organisms. For the first time, we will develop radiation protection standards that are based on actual risk."

This formed the basic philosophy for the DOE Low Dose Radiation Research Program, which was focused on using developments in technology and biology to evaluate changes in the low dose region. Sen. Domenici continued to provide support at a Gordon Research Conference on August 6, 1998, where he said, "I feel very strongly that we need the best possible standards for radiation risks, based on the best science we can produce."

DOE determined that by taking advantage of new technologies and techniques and combining them with the rapid advances in molecular and cell biology produced by the Human Genome Program, it might be possible to measure radiation-induced biological changes induced in the low dose region.

It was postulated that detecting changes at the cell and molecular level in the previously undetectable range below 0.1 Gy could now be done. For example, it was suggested that the extensive biological advances associated with sequencing of the genome, the development of gene expression arrays, and the expansion of information on cell-cell and cell matrix communication could be combined with technologies such as microbeams, systems that could expose individual cells to known types and amounts of radiation and measure biological responses in the low dose region. This approach could provide mechanistic data toward the development of a scientific basis for radiation standards in the low dose region. Such studies would make it possible for the scientific community and the public to evaluate the current standards to ensure they were adequate and appropriate to control radiation exposure during clean-up, waste storage, and use of nuclear power.

III. Development of the Program's Scientific Direction

Staff within BER drafted and developed the scientific directions for the Program. Dr. Frazier assigned Dr. David Thomassen as the Program Manager. In 1997, both Dr. Frazier and Dr. Thomassen were heavily involved in planning the first scientific meeting held to get input on the needs and scientific direction from the scientific community. Leading radiation biology scientists were chosen to form a subcommittee of the Biological and Environmental Research Advisory Committee (BERAC), a standing committee to provide advice to the DOE. This subcommittee was charged with developing a set of recommendations for DOE to use to develop the call for proposals for the new DOE Low Dose Radiation Research Program. The subcommittee was chaired by Dr. Robert Ullrich, and the committee members were:

- Dr. Robert Ullrich, Chair, Department of Radiation Oncology, University of Texas Medical Branch, Galveston, Texas
- Dr. Antone L. Brooks, Washington State University-Tri-Cities, Richland, Washington
- Dr. David Brenner, Columbia University, Center for Radiological Research, New York, New York
- Dr. Richard J. Bull, Pacific Northwest National Laboratory, Richland, Washington
- Dr. Eric J. Hall, Radiation Oncology Center for Radiological Research, Columbia University, New York, New York
- Dr. William F. Morgan, Professor of Radiation Oncology, University of California, San Francisco, San Francisco, California
- Dr. Julian Preston, Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina
- Dr. James Flynn, Decision Research, Eugene, Oregon
- Dr. Henry N. Wagner, Jr. Director, Division of Radiation Health Science, Johns Hopkins Medical School, Baltimore, Maryland
- Dr. Susan S. Wallace, Chair, Department of Microbiology and Molecular Genetics Director, Markey Center for Molecular Genetics, University of Vermont, Burlington, Vermont
- Dr. Gayle E. Woloschak, Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, Argonne, Illinois.

The executive summary of the BERAC report to BER (Appendix A) illustrates the general directions recommended made by the subcommittee during the scientific development of the Program. As the result of this and other meetings, a document was developed called "DOE-BER Low Dose and Dose-Rate Program" that provided the initial scientific questions, possible research areas, and preliminary suggestions for a potential budget. This document was helpful as the DOE staff drafted the first call for proposals from the scientific community and developed a budget for the Program.

The first call for proposals was then developed by BER's Drs. Ari Patrinos, Marvin Frazier, David Thomassen, and Arthur Katz. The doses to be investigated were set at levels below the exposure levels where risk can be derived using standard epidemiological methods. Thus, the Program focused only on the biological responses to low doses (<0.1 Gy, 10 rad) of low-LET ionizing radiation. Initially the focus was on the low-LET radiation of primary concern during waste clean up and nuclear power production. Later the Program was expanded to include high-LET radiation, as DOE and the National Aeronautics and Space Administration (NASA) combined programs to address problems of concern to both agencies. This expansion was to study high-LET radiation that would be encountered from high-Z particles and other high-LET radiation during space travel and the potential for exposure to high-LET radiation during waste clean up.

The primary goal in this first call for proposals was to develop a research program that built on advances in modern molecular biology and instrumentation not available during the previous 50 years of radiation biology research. The Program was to concentrate on understanding the relationships that exist between normal endogenous processes that deal with background oxidative damage and processes responsible for the detection and repair of low levels of radiation-induced damage. The research focused on understanding cellular processes responsible for recognizing and repairing normal oxidative damage and radiation-induced damage. The Summary and Supplementary Information from the first call (Appendix B) illustrate the early scientific directions that the Program established as a basis for evaluating the Program's success over the past ten years.

Proposals for research were received and scientifically reviewed from this call, and projects addressing all five major areas of concern were funded. The initial projects funded can be viewed on the website that was developed from this initial funding and continues to be funded at PNNL (http://lowdose.energy.gov/). Dr. Antone L. Brooks was selected as the lead scientist, and a committee was formed to provide direction and overview of the program. Dr. Thomassen was the Program manager until 2001 when he received another assignment in DOE. Dr. Noelle Metting, a radiation biologist and radiation physicist from Pacific Northwest National Laboratory who had joined DOE was assigned as the new Low Dose Research Program manager. Dr. Metting has been the manager since then and has provided guidance and direction to the Program over many years.

The remainder of this book is on the progress and scientific developments that resulted between 1998, when the funding started, to 2008. The Program has provided extensive scientific data on the responses to low doses of ionizing radiation and continues to explore the mechanisms behind these responses. Current research is directed toward important questions that remain to be addressed to make the data more useful in the regulatory arena.

Chapter 4

Early Observations and New Technology

The DOE Low Dose Radiation Research Program was founded on the presumption that it would be possible to use techniques and technology developed as part of the Human Genome Project to measure, characterize, and understand biological responses produced by exposures to low doses of radiation. Two major areas needed additional tools to make these measurements.

The first was dosimetry. It was not possible at low doses, where only a small fraction of the cell population had energy deposited in it, to determine which cells were "hit" and had energy events in them and which cells were not hit. Thus, in the low dose region, a technique was needed not only to know which cells had energy deposited in them, but what the cells' response was, down to single traversals from ionizing radiation, the lowest dose possible to a cell. The microbeam represents such a technique.

The second was the area of sensitivity of the biological response to low doses and the time required to measure changes in the low dose region. In the past, most cellular and molecular responses to radiation required large doses to be delivered to the cell population to make meaningful measurements. Such measurements required a large investment of time and money. The Human Genome Project and other programs being conducted by funding agencies including NIH and DOE developed a number of methods that were very rapid, had the needed sensitivity to measure radiation induced biological changes in the low dose region. These techniques were used by researchers in the DOE Program and made it possible to generate useful data in the low dose region of interest to the Program. All these new technologies were used by researchers in the Program and generated very useful information and data on how molecules and cells respond to low doses of ionizing radiation.

I. Development of the Microbeam

One of the most important tools developed by the Program was the microbeam, which made it possible to do the ultimate low dose studies in which single cells could have energy deposited in them, and their responses and those of their neighbors could be studied. The Program played a central role in the development and use of microbeams, which combine physics and biology to better understand the response of individual cells to radiation.

Using microbeams, the type of radiation, the total energy deposited, and the number of particles deposited in identified cells can be altered. The response of the "hit" cell and of the neighboring cells can then be studied. From such studies it has been demonstrated repeatedly that the "hit" cells as well as "non-hit" neighboring "bystander" cells respond to ionizing radiation. Such observations have caused a shift away from the major paradigm that only cells with radiation deposited in them respond to radiation.

Microbeams were designed to accurately and rapidly expose individual cells to known amounts of energy and to a wide range of different radiation types (alpha particles, X-rays, protons and electrons) (Nelson et al. 1996; Folkard et al. 2001a; Randers-Pehrson et al. 2001; Wilson et al. 2001; Resat & Morgan 2004b). The development of this equipment paved the way for many more studies on bystander effects, demonstrating the importance of both direct cell-to-cell contact and communication through the release of substances from the hit cells that influenced the responses in the non-hit cells.

As more institutions through the world developed and used microbeams, regular international meetings were held each year to compare research and further develop these important machines. Publications and summaries from these meetings indicate the wide range of important studies that were enabled by microbeam technology. A publication of the abstracts in 2006 in the journal *Radiation Research* 166: "Proceedings of the 7th International Workshop: Microbeam Probes of Cellular Radiation Response" is a good example of the variety of microbeams developed and the type of research questions that would be addressed with these new technologies.

A. PNNL-Texas A&M

An alpha particle microbeam was developed at PNNL (Braby 2000) by combining an accelerator, which provided helium particles of known energy, with a focusing magnet to place the alpha particles on a known spot and a microscope to locate the biological target. A diagram of this machine is shown in Figure 2. The basic parts used in this microbeam were similar to those used elsewhere.

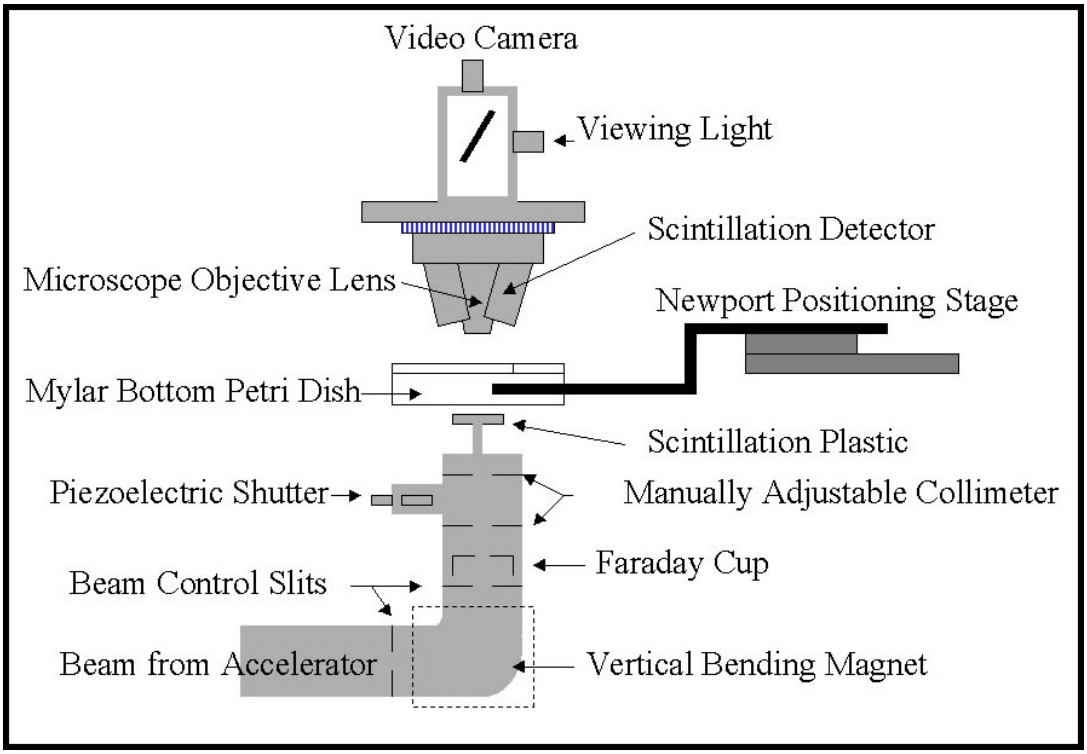


Figure 2. Schematic of an alpha-particle radiation microbeam showing how individual cells can be "hit" by known numbers of alpha particles.

The first biological studies were conducted with this microbeam at PNNL. These made it possible to relate the response of hit cells and the number of hits/cell to one biological response, the induction of micronuclei. This facilitated the generation of dose-response relationships for the induction of micronuclei (Nelson et al. 1996). These dose-response relationships were compared to the response to the same endpoint following exposure to uniform alpha irradiation from ^{239}Pu sources (NRC 1999) and to exposure to radon gas (Brooks et al. 1997). These comparisons made it possible to relate the number of hits per cell to the response to defined doses from uniform alpha sources (Brooks et al. 1994; Miller et al. 1996; NRC 1999). This demonstrated for the first time that cells hit with multiple alpha particles can survive and have no visible micronuclei induced. At the time that these studies were conducted, it was not recognized that both hit and non-hit cells could respond to alpha particle radiation.

At Texas A&M, the alpha particle microbeam was used to make several important scientific observations. For example, studies were conducted to determine if "hit theory" could be used to relate dose to biological response following exposure to alpha particles. Studies were designed to help define the relationships between radiation dose and hit numbers. These studies suggested that the number of cells hit may not be the most meaningful parameter to be used to relate alpha exposures to biological responses (Braby & Ford 2000).

This microbeam was used with the well-established rat trachea model to determine how cell-cell communication is triggered by alpha particles using intact tissue. These studies tested the hypothesis that normal respiratory epithelial cells transmit signals to neighboring cells in response to a small fraction of the cells being hit or a very low dose radiation exposure. Energy patterns were varied and the induction of bystander effects measured in a series of studies to determine what parameters were necessary to produce bystander effects (Braby & Ford 2004).

Finally, the research team also conducted studies to determine if the bystander effects observed following alpha particle microbeam irradiation were an artifact produced by the preirradiation of the surfaces of the cell culture material (Medvedeva et al. 2004), These studies suggested that in some systems, bystander effects may be related to radiation-induced changes in the tissue culture surfaces and not to a cellular response. The potential for such artifacts require further investigation and must be carefully controlled to make microbeam studies meaningful. These artifacts were not present in many other systems and bystander effects do exist, cells directly communicate with each other to produce damage in non-exposed cells, and they release biologically active substances into the media that can alter the response of non-exposed cells.

After the alpha microbeam was moved to Texas A&M University in 1997, an additional electron gun microbeam was developed at PNNL (Resat & Morgan 2004b). The electron gun microbeam is shown in Figure 3.

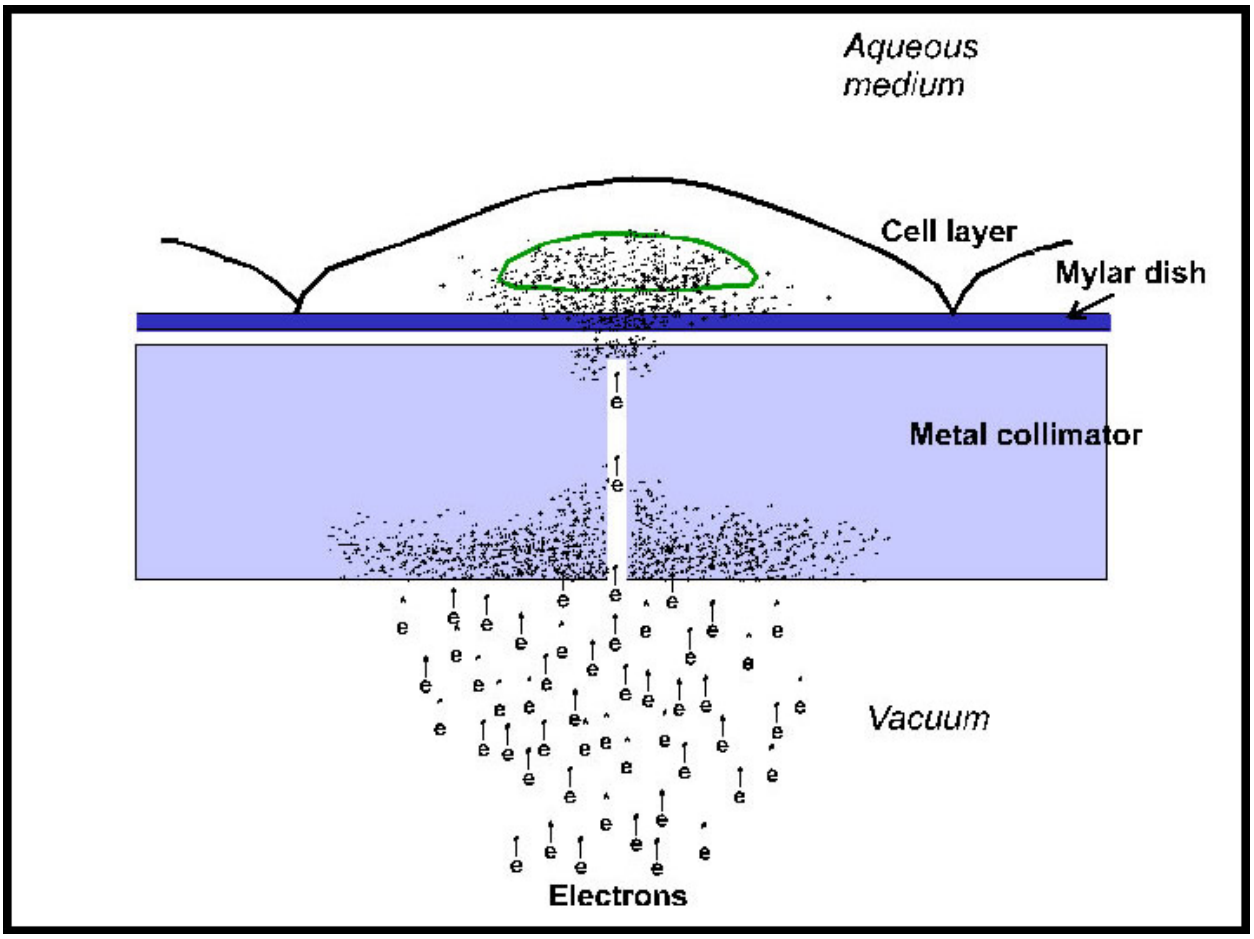


Figure 3. A spatially resolved electron gun microbeam with a cell in place to define dose distribution. Once the electrons go through the collimator, they scatter and result in a distribution of energy within the cell.

This microbeam used a focused electron beam, so it provided information on cellular responses to low-LET radiation delivered to known cells and locations (Resat & Morgan 2004a; Resat & Morgan 2004b). Extensive research was conducted on this machine to characterize the beam size, energy distribution, and penetration depth, and other characteristics of the energy deposition in cells (Wilson et al. 2001; Resat & Morgan 2004b; Lynch et al. 2005).

This device consisted of a pulsed electron beam capable of operating at energies from 20 to 80 keV. The electron gun is housed in a standard vacuum chamber pumped by a turbo molecular pump (base pressure 1 x 10^{-8} Tor). An electron source provides a beam with selected energy in the range of about 50 to 100 keV and fluence of about 3 x 10^{10} electrons cm^{-2} s^{-1} to a collimator chosen for the specific experiment. The chamber is equipped with a Faraday cup for monitoring beam current and an optical shutter to ensure no electron dark current between pulses. The spatial resolution of the device is achieved by passing the electron beam through a high aspect ratio hole (~20:1) fabricated in a metal foil (the collimator). Several collimators were designed with the goal of minimizing the production of X-rays while optimizing the spatial resolution of

the delivered dose. The beam collimation holes (typical diameters of ~5-15 mm) are formed using laser drilling. The collimator can be constructed with one hole or a series of holes depending on the biological experiment of interest (Resat & Morgan 2004b; Sowa et al. 2005).

This equipment has been very useful in measuring important track structure variables associated with the exposure of cells to electrons (Wilson et al. 2001). This instrument has also been important in determining the role of low-LET radiation in production of biological alterations in cells that did not have energy deposited in them. Such studies suggested that alpha particles were more effective in initiation of cell-cell communication than exposure to low-LET radiation. Such communication was essential for induction of bystander effects or biological alterations in non-hit cells. Some studies with low-LET microbeam radiation supported this observation as they failed to demonstrate the initiation of cell-cell communication and bystander effects (Morgan & Sowa 2005).

As the Program direction changed, many of the later studies moved from the response of individual cells in monolayer tissue culture to studying the responses in complex tracheal tissues (Ford et al. 2005). At Texas A&M, tracheal tissue was irradiated with a highly collimated electron microbeam irradiator or with a single-particle positive ion microbeam irradiator. This made it possible to compare the responses in single-cell tissue culture systems with the responses in normal rodent respiratory tissue cells. Such studies helped provide a method to extrapolate responses in tissue culture to the responses of human respiratory epithelial cells after exposure to a variety of radiation types. These studies have been critical in linking cellular and molecular studies to human risk.

B. Gray Cancer Institute

The Gray Cancer Institute (GCI) in England had also developed an alpha particle microbeam before the start of the Program and used it to study the interaction of alpha particles with hit and non-hit cells (Prise et al. 1998). Studies from this institution were funded by the Program and suggested that microbeams provide a unique tool to help understand the response of individual, identified cells to the exposure of known numbers of alpha particles (Michael et al. 2001; Folkard et al. 2002; Prise et al. 2002).

The other major development at the GCI was the development of low-LET microbeams using X-rays. The GCI pioneered the use of X-ray focusing techniques to develop systems for micro-irradiating individual cells and sub-cellular targets (Schettino et al. 2000; Folkard et al. 2001b; Folkard et al. 2001a; Michael et al. 2001). The prototype X-ray microprobe was developed alongside the existing charged-particle microbeam to address problems specific to low-LET radiations, where very precise targeting accuracy and energy delivery are required. A diagram of this microbeam is shown in Figure 4.

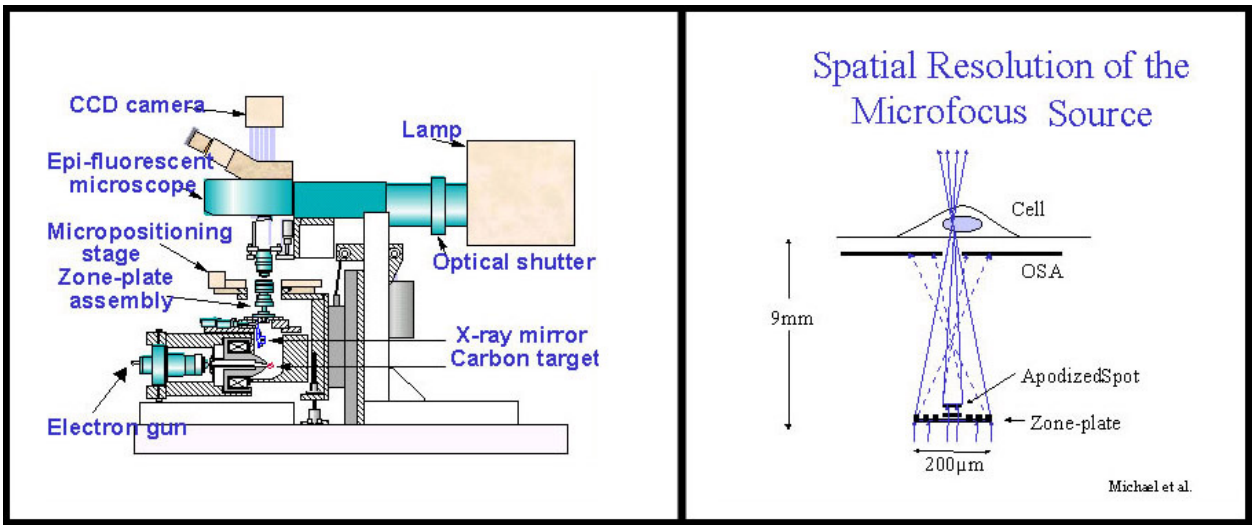


Figure 4. Schematic of a focused X-ray microbeam that illustrates spatial resolution of individual cells hit by X-rays.

The X-rays were generated by bombarding a target with energetic electrons. This generates so-called “characteristic X-rays” whose energy depends on the target material. Using this machine it was possible to radiate one cell at a time with defined doses and with sub-micron precision. The X-rays are then focused on a very fine spot (smaller than an individual cell) using a “zone-plate,” which is a small lens (less than 1 mm diameter) of a type developed initially for X-ray microscopy. To increase the energy is simply a matter of choosing other target materials; for example, aluminum or titanium instead of carbon. It thus became possible to use very low doses approaching that of a single electron track deposited in a single cell.

This microbeam was optimized for focusing 278 eV CK X-rays; however, there are a number of reasons for extending the range of available energies. To do this, a variable-energy soft X-ray microprobe was developed that was capable of delivering focused CK (0.28 keV), AlK (1.48 keV), and notably, TiK (4.5 keV) X-rays. TiK X-rays are capable of penetrating well beyond the first cell layer (the 1/e attenuation in tissue is 170 µm) and are therefore much better suited to studies involving tissues and multi-cellular layers. Also, from a microdosimetric point-of-view, TiK X-rays produce a spectrum of energy depositions in DNA-sized targets that more closely resemble those of conventional low-LET radiations (Schettino et al. 2000; Folkard et al. 2001b). This type of exposure is very relevant to environmental levels of exposure.

Such research made it possible to concentrate on irradiating specified individual cells within cell populations to identify bystander responses for low-LET radiation where non-radiated cells respond to signals from nearby radiated cells (Schettino et al. 2003). Modification of the equipment made it possible to use higher energy X-rays to extend the studies into complex tissues and beyond experiments involving single cell layers. These types of microbeams generate types of low-LET radiation that more closely mimic the types of exposures of prime interest to DOE during waste clean-up as well as the type of radiation received by nuclear workers or during nuclear accidents.

C. Columbia University

Columbia University was also among the first to develop an alpha particle microbeam (Randers-Pehrson et al. 2001) and was the first to develop a microscope system to automate the location of cells. This was done by combing a scanning microscope stage with the use of a vital dye that stained the nucleus. Using this system, it was possible to locate and irradiate large numbers of cells very rapidly to a defined number of alpha particles. This made it possible to study the influence of defined numbers of alpha particles on cell transformation response in bystander C3H 10T1/2 cells (Miller et al. 1999; Sawant et al. 2001b).

Columbia scientists also developed special staining techniques to identify different cell types grown in the same dish. This culture technique was combined with the microbeam. A known numbers of alpha particles could hit cells stained with one type of dye while the other cell type did not have energy deposited in them. The number of micronuclei could be scored in both the hit cells and the non-hit cells and hit-response relationships derived in the different cell types on the same dish (Ponnaiya et al. 2004). The frequency of micronuclei was observed to increase as a function of the number of alpha particles that traversed the hit cells as was expected. It was also demonstrated that the non-hit cells also had more micronuclei that the controls which represented a direct demonstration of the bystander effect. In Figure 5, both hit and non-hit cells are shown to have micronuclei in them.

The scientists at Columbia University also developed a novel co-culturing technique where irradiated and bystander cells were cultured on two surfaces of Mylar separated by media (Geard et al. 2002). Because the range of the alpha particles was short relative to the distance between the cells, the cells on one surface could be irradiated, and cells on the other would receive no energy deposition. Using this technique, it was possible to irradiate large cell populations to investigate the induction of chromosomal aberrations in irradiated and bystander immortalized human fibroblasts. Using this system, it was possible to show that bystander effects were present in the non-irradiated cells.

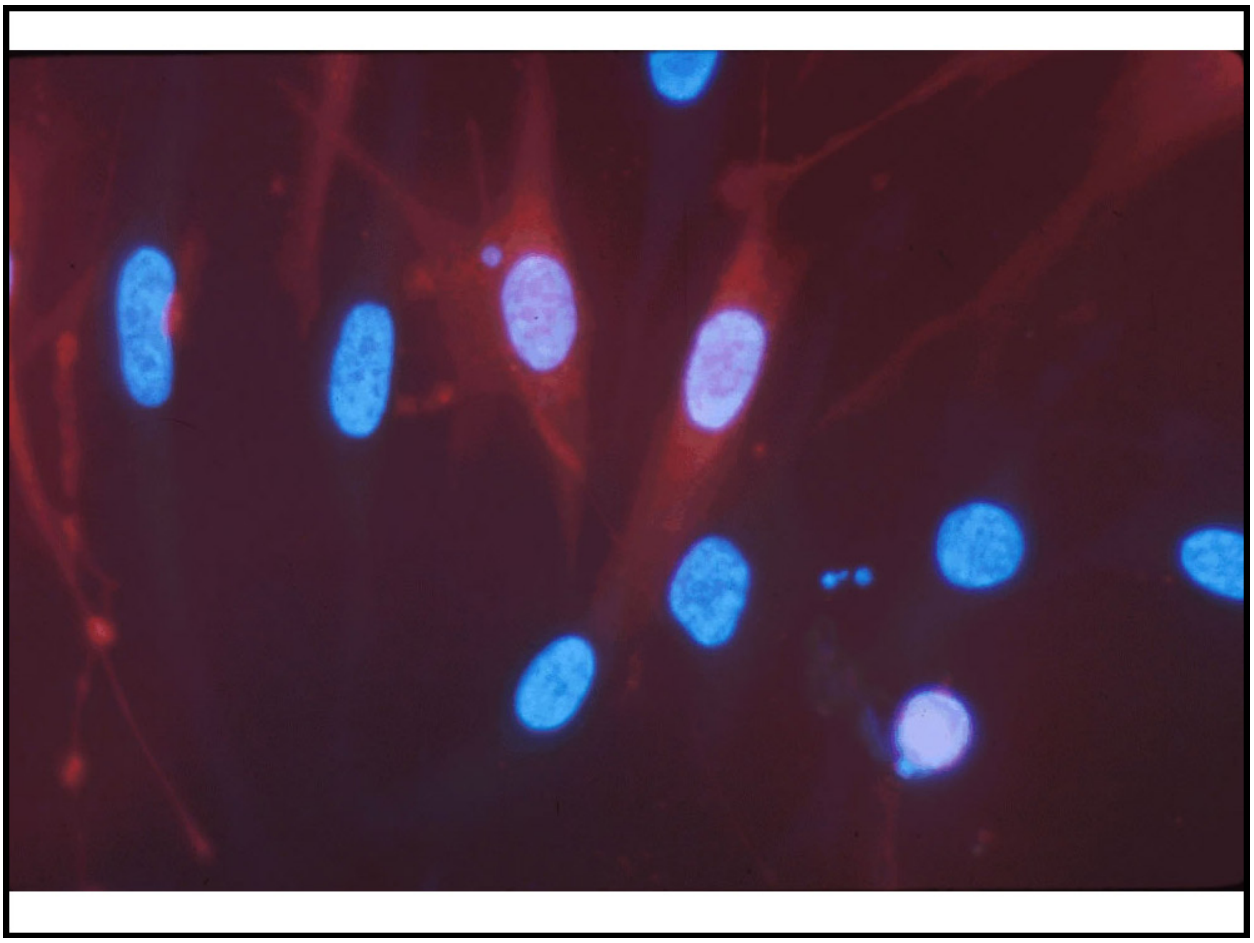


Figure 5. Stained cells showing cells hit (light blue) and not hit (dark blue) by microbeams, demonstrating that micronuclei in the non-hit cells can be damaged as a result of the bystander effect (Ponnaiya et al. 2004).

Columbia scientists also demonstrated that there was a well-defined dose-response relationship in the cells directly exposed to alpha particles and that the type of aberrations observed were, as would be expected, chromosomal. However, in the bystander cells there was no dose-response relationship, and the level of aberrations remained elevated above that seen in the controls at a constant rate. In addition, the type of aberrations observed in the bystander cells were the chromatid type, which would not be predicted with cells in the stage of the cell cycle used in this study. Such studies demonstrate that the bystander response is unique and may have different significance in risk analysis

D. Lawrence Berkeley National Laboratory

Lawrence Berkeley National Laboratory (LBNL) also developed a type of a microbeam using an X-ray microprobe at the Advanced Light Source (ALS) to precisely irradiate individual cells and specific regions in cells without damaging neighboring cells. The unique synchrotron-based source of a 12.5-keV X-ray microbeam line 10.3.1 at the ALS was used to quantitatively

characterize low-dose responses of low-LET, radiation-induced bystander effects in a novel tissue-like model of human mammary epithelial cells (HMEC), normal human fibroblast cells (HFC), or in a third scenario, with both cells together in a co-culture system (Mainardi et al. 2004). Cultures were grown in microwell slide chambers and irradiated with precise stripes of radiation that were up to 100 μm wide. An example of exposed and non-exposed cells is shown in Figure 6.

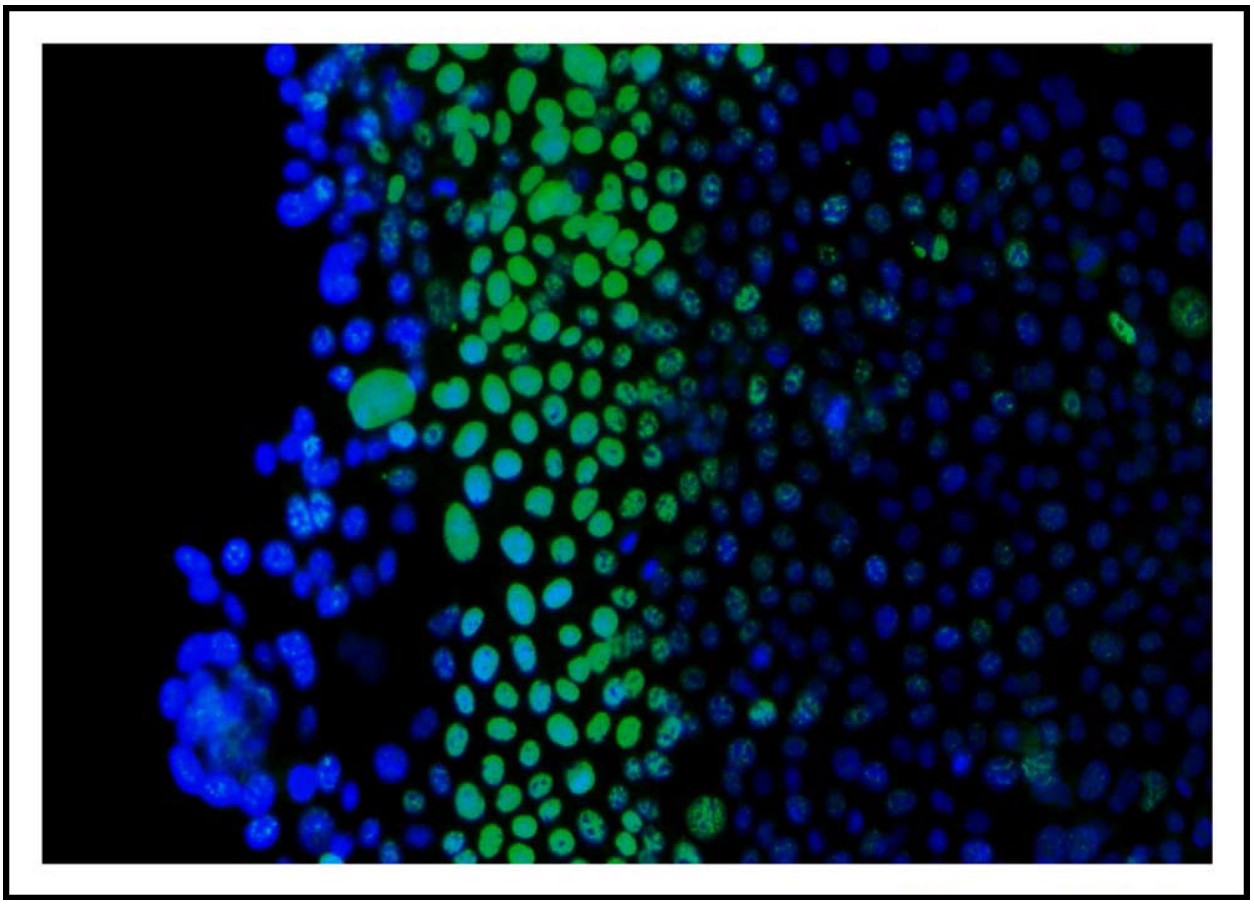


Figure 6. Cells in culture irradiated with a strip microbeam. Green cells were hit, blue cells were not. Figure illustrates the bystander effect in that the signal migrates from the hit to the non-hit cells to produce damage, as seen by the scattered green cells. (Blakely et al. 2006).

With this system, a group of cells in a defined stripe could be irradiated. The response in these cells was then compared to those outside the stripe that received no radiation energy. Samples were processed for the expression of radiation-induced protein markers with fluorescent immunohistochemistry in a time course from 10 minutes to several hours after exposure. Using fluorescence microscopy on a high-precision-controlled microscope stage and fiducially marked references, the physical locations of the dose stripes were mapped exactly to the location of the biological responses. Computer-based fluorescent analysis of radiation-induced signals in thousands of cells has revealed statistically significant differences in the broadening of the effects of the dose stripes to neighboring unirradiated cells with time after exposure. Such

broadening of the dose stripe to involve cells not in the irradiated field represented a radiation-induced bystander effect that was quantitatively evaluated.

The sensitivity of detection in this model system is below 0.1 Gy, with dose stripes discernible after 0.05 Gy. The intensity of the fluorescence was greater in the dose stripe for larger doses (e.g., 1.0 Gy), and the fluorescence signal decreased more slowly with time after high-dose exposure than after lower doses (e.g., 0.25 or 0.1 Gy). Results from a rapid time course study show that radiation-induced signals were observed within 10 min after exposure in cells adjacent to, but outside of the irradiated area. The effect was apparent at 10 min after exposure and diminished with time, but was still significant 3 h after exposure. A dose-dependent induction of bystander effects in several classes of radiation-induced signals was measured and the time course determined to examine how radiation exposure changes cell signaling acutely, and chronically (Blakely et al. 2006).

With the development of the microbeams and the discovery and characterization of the bystander effects, it was no longer adequate to think in terms of individual cell responses as a model for radiation risk estimates. Well-established paradigms in radiation biology have been challenged including the hit theory and the use of dose to cells as a means of predicting radiation responses. With these new observations, a broader "systems" type of thinking is required in which the whole tissues or organs respond to the radiation exposure in a coordinated way.

II. Biotechnology

Other important techniques were developed that made it possible to conduct research in the low dose region and to evaluate the biological responses. These techniques are discussed briefly in this chapter, and the responses and significance of the science is covered in subsequent chapters.

A. Flow Cytometry and Chromosome Painting

The flow cytometer was developed primarily at LANL using a combination of rapid flow of liquids and laser technology. Cells were suspended in a suitable liquid that would fall rapidly, forming individual drops that passed in front of a laser. The laser would determine if each drop had a cell in it and measure traits associated with the cell. Using an electric field and magnets, it was possible to charge the drops with the cells of interest and deflect the drops and collect them in individual containers. This technique made it possible to sort individual cells, chromosomes, and organelles well before the Program began (Wimmer et al. 1996; Cram 2002; Cram et al. 2002). This technique is an important part of research in many areas, including the research on health effects of radiation (Wilson & Marples 2007). Program researchers have used flow cytometry to evaluate many cellular and molecular changes induced by ionizing radiation in populations of cells.

An important spin-off from the flow cytometer is the development of whole chromosome paints. Using flow cytometry, individual chromosomes could be sorted from the remainder of the genome and probes developed that were specific to each chromosome. These probes made it possible for each chromosome to be "painted" a unique color. Probes for parts of the chromosome and individual genes were further developed by the Human Genome Project, which made it possible to determine the exact location of genes on each chromosome. With the ability

to mark each chromosome with whole-chromosome paints, it has become obvious that each chromosome has a unique domain in the cell nucleus during all stages of the cell cycle (Cornforth et al. 2002a; Vives et al. 2005). It was determined that at metaphase, each of the individual chromosomes involved in radiation-induced damage can be accurately identified.

Early dose-response studies conducted in the program without using chromosome painting could not determine the number and types of chromosomes involved in radiation-induced aberrations. In studies such as those where cells or animals were exposed to high-Z particles similar to those found in space (Brooks 2001; Brooks et al. 2001), many of the complex aberrations were scored as single exchanges, and many of the complex exchanges were not detected. Later, more complete studies using these paints were conducted that made it possible to determine the involvement of each individual chromosome in the chromosome aberrations (Cornforth 2001; Vazquez et al. 2002). With Fluorescent In Situ Hybridization (FISH) techniques, it became possible to do very complete dose-response curves (Loucas & Cornforth 2001) and evaluate the individual chromosomes involved (Cornforth 2001), the number of chromosomes that make up each aberration, and the location within each chromosome where the aberration is induced. An example of a chromosome spread that is painted with these techniques and a dicentric and fragraments produced by radiation are shown in Figure 7 (Loucas & Cornforth 2001; Cornforth et al. 2002a).

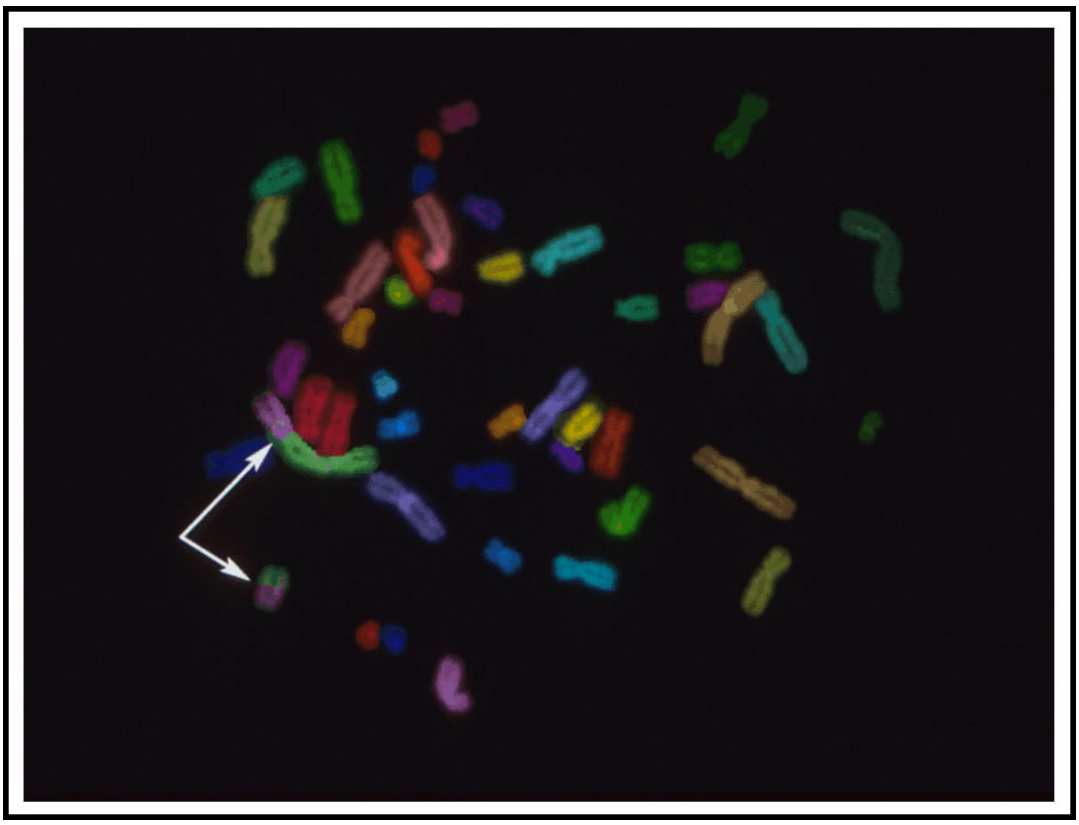


Figure 7. Chromosomes visualized with fluorescent in situ hybridization (FISH). Arrows show a simple dicentric interchange between chromosomes 2 and 8, resulting in two color junctions. (Cornforth et al. 2002b).

These staining techniques required the development of different systems of scoring chromosome aberrations (Tucker et al. 1995; Cornforth 2006). Chromosome painting demonstrated that many of the aberrations that were scored in the past with conventional techniques as simple exchanges involving two chromosomes, actually involved multiple different chromosomes (Cornforth 2001; Vazquez et al. 2002). Such studies have been extended to determine the influence of dose-rate (Loucas et al. 2004b) and radiation-type including gamma rays, and high-Z particles on the induction of complex chromosome aberrations (Cornforth et al. 2002b; Loucas et al. 2004a; Rithidech et al. 2007a). The frequency, distribution, kinetics of repair, and type of complex aberrations have all been characterized as function dose, dose-rate, and LET.

An additional technique, Co-FISH, was developed using a combination of the staining and sorting techniques that made it possible to label one set of DNA in a cell, resulting in one chromatid being labeled, but not the other (Bailey et al. 2001a; Bailey & Goodwin 2004; Zou et al. 2004). This technique was very important in understanding the function of telomeres and their role in the formation of chromosome aberrations and DNA repair (Bailey et al. 2004c; Bailey et al. 2004a; Bailey & Cornforth 2007). These additional data on the function of telomeres may become important in the future in estimating the role of radiation on the development of disease. The role of flow cytometry in study of radiation biology is critical and has been carefully reviewed (Wilson & Marples 2007).

B. Gene Chip Technology

1. Genomics

As was expected at the start of the Program, the development of gene chip technology as part of the Human Genome Project and the field of genomics proved to be very important in understanding the biological responses to low doses of radiation. This tool made it possible to rapidly evaluate the radiation-induced changes in gene expression, protein production, and changes in metabolites following any environmental insult, including low doses of radiation. Application and modification of this technology was an important part of the Program (Bittner et al. 2000; Koch-Paiz et al. 2000; Kegelmeyer et al. 2001).

With gene chip technology, it became possible to identify many radiation-induced changes in gene expression for thousands of genes at one time and to determine which genes were either up or down regulated as a biological response to exposures to low doses of radiation. Because of the large amount of data generated by such approaches, it was also necessary to develop additional informatics methods to handle and to interpret such data (Fornace et al. 1999).
In many early studies, cells were simply exposed to low doses of radiation and the changes in gene expression determined (Amundson et al. 2001c, b; Yin et al. 2003).

The type of genes that responded to low doses of radiation could also be defined. Early studies quickly determined that many of the genes that changed their gene expression following low doses of radiation were the same genes that respond to many other forms of stress (Amundson et al. 1999; Amundson et al. 2001c; Amundson et al. 2002; Amundson & Fornace 2003; Amundson et al. 2003).

As with any new technology, this technology has great possibilities but there are always areas where data generated by such a broad-based approach can be misinterpreted (Amundson & Fornace 2003). Contrary to expectations, initial data did not suggest that low doses of radiation had marked impact on the expression of genes known to be associated with DNA repair (Kegelmeyer et al. 2001; Yin et al. 2003; Akerman et al. 2005). As gene chip technology was applied to evaluate the radiation response as a function of time after radiation exposure (Amundson et al. 2002; Amundson et al. 2003), radiation dose (Yin et al. 2003; Ding et al. 2005; Coleman & Wyrobek 2006), dose rate (Amundson et al. 2003), and radiation type (Kurpinski et al. 2009), it became clear the gene responses were very complicated and were modified by all these factors (Amundson & Fornace 2003).

The take-home message of these early studies was that the genes that responded at low doses and dose rates were different than the genes that responded after high doses. This suggested a difference in the mechanisms of action following low dose radiation compared to those following high doses (Coleman et al. 2005; Ding et al. 2005; Brooks et al. 2006b; Coleman & Wyrobek 2006). Such a difference suggested that the shape of the dose-response relationship could be non-linear. It was also clear that the time course of the response and the shape of the dose-response relationships were different for many individual genes. These early gene expression studies provided the groundwork for many more mechanistic studies of the observed radiation-related processes, such as changes in reactive oxygen species status of the cells, bystander effects, adaptive responses, and genomic instability discussed in greater detail in Chapters 5 and 6.

2. Proteomics

Gene expression and protein expression are not linked 100%, and many of the important proteins involved in biological changes do not follow the changes in gene expression. Many biological processes are altered by changes in protein expression. These may be related to time differences, differences in breakdown and up regulation of the genes and proteins, differences in intercellular location of the proteins (Raman et al. 2007), and other factors. The use of proteomics in biology is relatively recent in the Program and is currently being more widely used.

Many recently developed techniques make the use of proteomics possible. Development of chips similar to gene chips was a major technical advance. With such techniques, it became possible early in the Program to clone and characterize known proteins in mice and humans (Coleman et al. 2000). These protein microarrays also made it possible to define many different protein interactions with cellular components such as the chromatin in the cells (Coleman et al. 2003a). As better multiplexing techniques were developed, it became possible to identify bacterial and viral proteins in mammalian protein samples (Rao et al. 2004), which could be very useful in the future to identify and diagnose diseases.

It was demonstrated that many cells could shed proteins as the result of environmental insults including radiation exposure. By applying proteomic techniques, (Ahram et al. 2005a) determined that these shed proteins could be characterized into different classes. By combining the proteomic approach with the databases that have been developed as the result of proteomic

research, some of the shed proteins were identified in Chinese hamster ovary (CHO) cells following radiation exposure (Ahram et al. 2005b).

Proteomics techniques have continued to improve by combining liquid chromatography/tandem mass spectrometry (LC-MS/MS) with other methods of isolating and characterizing proteins. The speed of processing samples with these techniques has been a limiting factor, but the number of samples processed has been increased by using high-intensity focused ultrasound in sample preparation in combination with LC/MS techniques (Lopez-Ferrer et al. 2008). Such techniques have also used much faster methods based on shared peptides to identify the proteins (Jin et al. 2008). These techniques hold great promise for linking the changes in gene expression to the proteins carrying out the biological functions.

The goal of all proteomic research is to link protein changes to biological function. It has been demonstrated that there are many post-translational modifications of proteins that are very important in determining the function of the proteins. Such modifications can alter the potential impact of the proteins in both positive and detrimental ways (Warters 2002). A major important change impacting the protein function is the phosphorylation of proteins. Extensive research in this area has been conducted, but only limited research in the Program. However, Program research has determined that it is possible to identify the phosphoproteome, which defines post-translational phosphorylation of the proteins and supplements proteomics. This research demonstrated that the phosphorylation of proteins following radiation exposure to high doses was different from that observed after low doses of ionizing radiation (Yang et al. 2006).

(Yamaguchi et al. 2005) identified substrate specificity for human protein phosphatase 2Cδ, Wip1 as an example of how changing phosphorylation can change function. This made it possible to develop a substrate-based cyclic phosphopoptide inhibitor of this protein and led to many developments in identifying protein function and its modification. This research is continuing under the Program.

The ultimate goal of proteomics research is to relate molecular and cellular changes to well-defined biological changes as well as to exposure conditions. For example, Wang and Gao (Wang & Gao 2005) determined that proteomic analysis was useful in the study of neural differentiation of mouse embryonic stem cells to neurons. Such analyses can be applied to define any differentiation pathway as well as responses, as cells differentiate in a unique way as a function of radiation exposure.

Change as a function of radiation dose was the first exposure condition to relate protein profile changes with radiation exposure. These studies determined which and how much proteins changed as a function of radiation dose. Such information could then be used to estimate radiation dose where no physical dosimeters were present (Marchetti et al. 2006). This is a good example of applying Program-funded research in an area that was not an emphasis of the Program; in this case, biodosimetry. This is discussed in greater detail in Chapter 6.

(Jang et al. 2007) used a proteomic approach to relate specific changes in salmodulin. The relationships that exist between calcium- and phosphorylation-dependent calmodulin complexes were defined using such an approach and pave the way for more extensive studies on how

radiation can modify these relationships. These studies are laying the groundwork for further mechanistic studies that will be useful in defining many cellular processes triggered by radiation.

Signaling and chronic inflammation are discussed in great detail in Chapter 6. These processes can be studied and provide important links between the proteome and specific molecular mechanism that will be critical in using systems biology to better understand radiation risk (Wemer & Haller 2007).

A few studies have demonstrated that a proteomic approach can be useful in studying cancer and the processes that are important steps in cancer development. The mitochondria are the powerhouse of the cell and play a critical role in the generation of free radicals similar to those generated by radiation. (Miller et al. 2008) demonstrated that it is possible to use MS-based proteomic techniques to profile mitrochondrial proteins in radiation-induced genomically unstable cell lines. These unstable cell lines demonstrate a persistent oxidative stress and are thought to represent an important stage in the development of cancer.

A limited number of studies used animal models to study the role of protein changes during cancer development. Studies of radiation-induced leukemia demonstrated that proteomic techniques can suggest relationships that exist between radiation exosure, protein changes, and cancer development (Rithidech et al. 2007a). Such animal-based models are essential to link radiation-induced cancer in animals to that in humans.

3. Metabolomics and secretomics

The analysis of the many products produced as the result of metabolism or secretion stimulated by an environmental insult is called metabolomics and secretomics. Techniques similar to those used in proteomics have been used to identify these molecules. Because many products are identified using these techniques it is important to develop techniques to sort and characterize the interrelationships between these chemicals (Patterson et al. 2008).

In metabolomics, the products are sampled either in the urine or the blood. Because both body fluids are easy to obtain, metabolomics can be used as a biodosimetric technique to estimate previous radiation exposure (Tyburski et al. 2008). To date, radiation-induced changes in metabolites are not sensitive enough to detect exposure to low doses of radiation and have not been a focus of the Program. However, studies using metabolomics to study radiation-induced cell killing suggest that it may be possible to identify the two major types of cell death, apoptosis and necrosis, in HL60 leukemia cells (Rainaldi et al. 2008). This type of research needs to be expanded to other cell types, especially normal cells *in vivo*.

Because the role of the microenvironment is important in the development of radiation-induced disease and the maintainance of normal organ function, it is also important to determine its role in the secretation of hormones or other substances into a tissue or organ. (Chen et al. 2008) evaluated the role of the microenvironment on the “secretome”. This is a new area of research that may represent an important part of the systems biological approach needed in the future for evaluating radiation risk.

C. Techniques to Detect and Characterize DNA Damage and Repair

DNA damage and repair play a central role in the induction of cancer. Characterization of genes, proteins and pathways involved in repair of radiation-induced DNA damage was one of the major areas in the field of radiation biology and was an important area addressed initially by the Program. Two major questions posed in the original Program outline were:

- Is the damage induced by ionizing radiation and the repair of that damage different from the endogenous oxidative damage and repair present during normal life processes? Addressing this question is important because high levels of oxidative DNA damage are produced and repaired daily in every cell in our bodies.
- Does this DNA repair extend to damage from ionizing radiation? When the Program began, the ability to measure DNA damage following radiation exposure was limited to very high doses of radiation (Rydberg et al. 1994). Thus, much of the past research in this area was not applicable to the new DOE Low Dose Radiation Research Program.

Research in this area resulted in the development of techniques that could measure DNA damage following low doses of ionizing radiation and determine the similarities and differences between radiation-induced damage and repair and DNA damage produced by normal processes. The distribution of DNA damage following radiation was very non-uniformly distributed in the DNA, with local sites having multiple different types of damage (locally multiply damaged sites, or LMDS) (Ward 1994). This observation formed an important base for Program research.

New techniques were developed at Brookhaven National Laboratory that made it possible to detect these multiply damaged sites following low doses of ionizing radiation (Sutherland et al. 2000a, b; Sutherland et al. 2001d). The basis of these techniques was to convert all of the DNA lesions to double-strand breaks (DSBs) (Georgakilas et al. 2002) then separate the DNA according to size (Sutherland et al. 2001d; Sutherland et al. 2003c), and using single-molecule laser fluorescence sizing (Filippova et al. 2003) quantify the number of breaks and the size of the DNA strands (Sutherland et al. 2003a; Sutherland et al. 2003b). Using this combination of techniques, it was possible to measure the clustered DNA damage sites and the size of the lesions following very low doses of radiation (Sutherland et al. 2002b) over a wide range of different types of radiation exposure (Sutherland et al. 2001c; Song et al. 2002; Sutherland et al. 2002b) and under different experimental conditions (Sutherland et al. 2001a). It was suggested that such damage sites were unique for radiation, and the distribution of the damage was dependent on the radiation type (Hada & Sutherland 2006).

This early research also suggested that radiation-induced damage was formed in clusters and was different from the random distribution of DNA damage produced by normal endogenous processes. It was determined that additional research was needed on the repair and processing of radiation-induced clustered DNA damage.

An important development in understanding the relationship between cells hit by radiation and the response to the energy deposited in the cell was the development of methods to detect DNA damage and repair sites. It was determined that histones were phosphorylated in response to DNA DSBs (Burma et al. 2001). This process generated sites called γH2AX sites—sites of phospholated genes as a reaction on DNA double-strand breaks (DSB)—that could be visualized

at the site of the initial energy deposition. These were thought to be a marker of the location of the DNA breakage (Al Rashid et al. 2005) and repair (Burma & Chen 2004). The γH2AX technique, developed by a number of laboratories outside the Program, was incorporated into a several Program studies and provided very useful data.

When the γH2AX technique was combined with microbeam studies, it was possible to see which cells were hit by radiation, how many hits had occurred on a given nucleus, and how long it took the cells to repair the damage and lose the γH2AX foci. An example of this type of study and the information generated is seen in Figure 8 where single nuclei were traversed by three alpha particles (Prise et al. 2002). The γH2AX technique was an important tool used to evaluate many of the new biological phenomena that were seen in the Program.

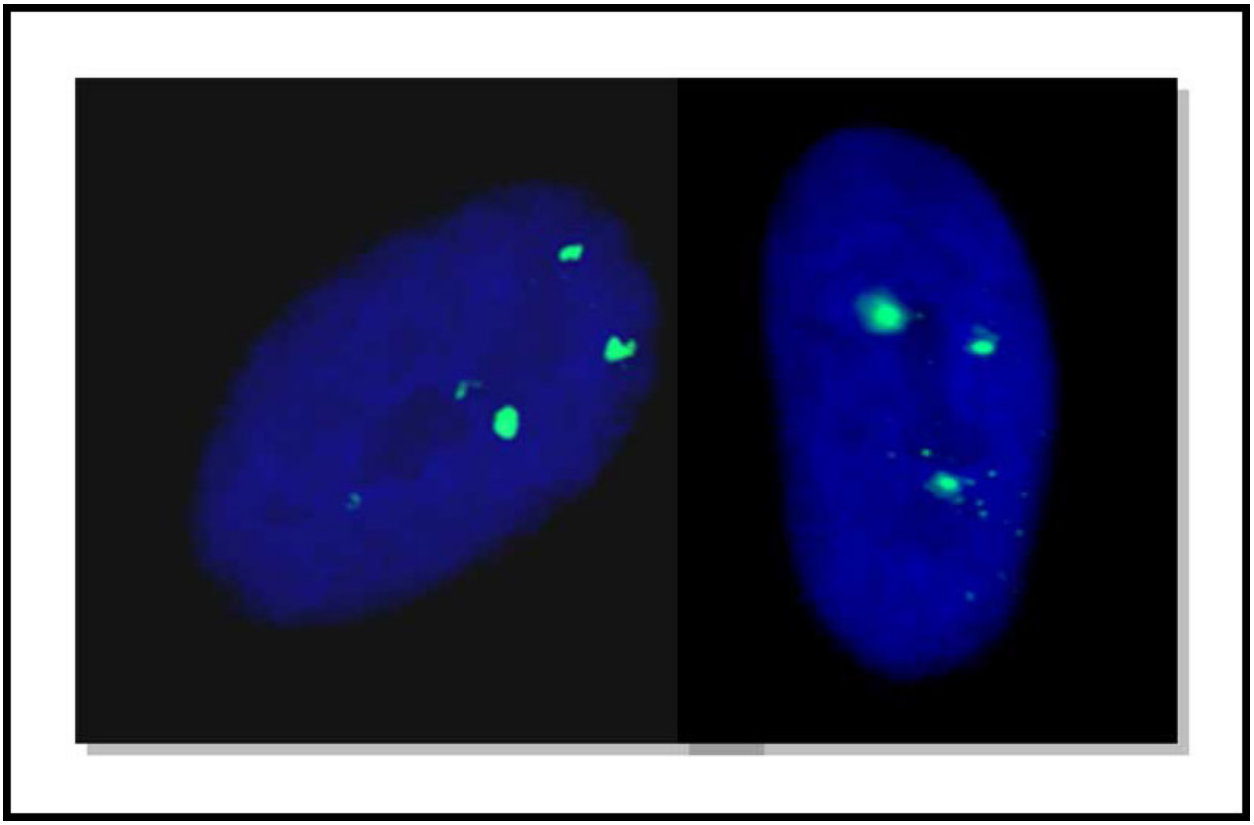


Figure 8. DNA damage is recorded as γH2AX foci in two nuclei of human fibroblasts targeted with the microbeam. Each nucleus was hit by three helium ions; a single ion in each of three locations, each helium ion delivered 100 mGy equivalent and produced four to six DNA double-strand breaks, shown in green (Prise et al. 2002).

D. Identification and Characterization of DNA Repair Genes

Extensive early research in radiation biology focused on the identification and characterization of many DNA repair genes, and under the Program, many more DNA repair genes were identified (Cleaver et al. 1999b; Blaisdell & Wallace 2001; Fujimori et al. 2001), characterized (Weinfeld et al. 2001; Lamerdin et al. 2004; Hirano et al. 2005), and defined. Interactions of the repair

genes with other genes were evaluated (Wiese et al. 2002; Miller et al. 2005), the pathways involved in the repair were defined (Pierce et al. 1999), and their roles in mutation induction (Shen et al. 1998), and maintaining genomic integrity (Cleaver et al. 1999b; Cleaver et al. 1999a; Hinz et al. 2007), following radiation damage were examined. The role of repair genes associated with radiation-induced DNA damage in regulating the genomic stability of cells and the induction of radiation-related genomic instability is discussed in greater detail in Chapter 6.

A major approach to understanding DNA repair has been to use model experimental systems, human families, populations, and cell types deficient in DNA repair to determine the genes and mutations involved in these deficiencies. Several major genetic diseases proved to be very useful in defining the genes involved in DNA repair and the role of these genes in the disease (Thompson & Schild 2002). The cells and tissues from individuals with genetic disease were exposed to radiation. The influences of radiation on many biological endpoints were determined in these deficient cells and populations, then were characterized and compared to normal responses.

A disease early recognized as resulting from a DNA repair deficiency was Xeroderma pigmentosum (Cleaver et al. 1999b; Cleaver et al. 1999a; Cappelli et al. 2000). Genes from this disease, model systems, and other diseases have been characterized and their role in DNA repair mapped and evaluated. Other syndromes of importance in defining DNA repair genes include Cockayne syndrome and trichotriodystrophy (Cleaver et al. 1999a), Fanconi anemia (Yamamoto et al. 2003; Tebbs et al. 2005; Thompson et al. 2005; Yamamoto et al. 2005; Hinz et al. 2006; Hinz et al. 2007), Nijmegen breakage syndrome(Williams et al. 2002; Pluth et al. 2008), and more recently, genes that influence the production of diseases such as breast cancer (Easton et al. 2007).

There were many publications in these same areas of research outside the Program that are not included in this book. These publications and those from the Program constructed a firm understanding of the role of radiation-induced DNA damage and repair and the consequence of that damage.

E. Cell Killing and Apoptosis

1. Cell killing

A major focus in radiation biology is cancer therapy research, thus cell killing was a hallmark of much of the early research. As it became possible to culture mammalian cells, methods were developed to determine the ability of the cells to divide and form colonies following radiation exposure. Because radiation did not easily kill many cell types in interphase but would prevent them from dividing and forming viable colonies, the colony formation method became the standard for evaluating radiation-induced cell killing.

To conduct this technique, a known number of cells were seeded in a dish, irradiated, and allowed to divide and form colonies. The colonies were counted, and the number in the exposed dishes compared to those in the control dish to estimate the radiation-induced cell killing. Cell survival curves were characterized for many different types of radiation exposure, different dose-rates, fractionation schedules, cell types, and tissue types. Normal and genetically altered cells

were studied to provide information on the role of many genes on the induction and repair of radiation-induced damage. The methods to conduct cell survival studies and the shape and slope of these survival curves have been carefully reviewed in the light of modern biology (Hall & Giaccia 2006). The use of survival curves was critical in the development of the hit theory, which needs to be revised in light of more recent data on cell survival.

High-LET radiation (alpha particles and neutrons) produced a linear decrease in survival as a function of dose when plotted on semi-log paper. After exposure to high-LET radiation, cell survival had only minor dependence on dose rate or dose fractionation, so the radiation response could be easily described and quantified. From such data, it was assumed that single hits were responsible for cell death, and there was little repair following this type of radiation.

However, following exposure to low-LET radiation (X-rays, gamma rays, and beta particles) there seemed to be a threshold dose range over which cell killing did not change from that observed in the controls. Following exposure to low-LET radiation, the response was decreased by reducing the dose-rate and allowing time for "recovery" by dose fractionation schedules. These data suggested that there was repair of the lesions and that multiple hits were required to kill cells. The width of this threshold or shoulder was a function of the cell type, radiation dose-rate, and radiation type. However, the colony formation technique was not sensitive enough to determine the fine structure of the dose-response relationship for low-LET radiation exposure in the low dose region of importance to the Program.

Studies conducted on the induction of damage from small doses *in vivo* in mouse skin (Joiner et al. 1986) and in mouse renal tissue (Joiner & Johns 1988) suggested that the effectiveness of small doses of low-LET radiation was higher per unit of dose in producing cell killing than larger doses. Determining this low dose sensitivity *in vitro* became possible after development of a dynamic microscopic image processing scanner (DMIPS) cell analyzer, which made it possible to locate each cell on a dish and, after exposure to radiation, directly measure the number of cells that survived and formed colonies (Marples & Joiner 1993). This technique made it possible to more accurately measure cell survival in the low dose region and added an important tool for use in study of low dose radiation effects. It also formed the basis for studies that determined that the apparent plateau in the low dose region was really an area where the cells were more sensitive per unit dose then became resistant as the dose increased. Thus, there was structure in the low dose region that was not appreciated in the past.

With the development of the flow cytometer, additional sensitive techniques for the detection of cell killing were developed (Short et al. 1999; Bogen et al. 2001). These techniques were faster and also provided information on the stage of the cell cycle during radiation exposure (Short et al. 2003) that helped define the mechanisms of action for this observed fine structure in the dose-response relationship.

It is important to provide a brief description of the fine structure in the dose-response relationships in the low dose region. At very low doses, there was a steep curve for cell killing called hyper-radiosensitivity (HRS), followed by a upswing in the survival over a narrow dose range, called increased radioresistance (IRR), and finally, as the dose continued to increase, the final slope of the dose response as detected by other less-sensitive techniques was evident

(Marples & Joiner 1993). Thus, the plateau over the low dose region was not a true plateau, but an area of low dose hypersensitivity (HRS) followed by radiation-induced resistance. This observation was made in many cell systems and seems to be a biological generality. Some of the cell types where HRS and IRR were measured and observed included human cells, cancer cells, and immortalized cell lines from humans and animals (Short et al. 1999; Mitchell et al. 2002; Chalmers et al. 2004; Harney et al. 2004b; Harney et al. 2004a).

2. Apoptosis

Another very useful area of rapid development in cell and molecular biology in the Program was the increased understanding of programmed cell death (apoptosis). This field has expanded rapidly and is becoming very important in cancer therapy. Detailed information on apoptosis and its role in cancer induction and therapy can be found at http://www.apoptosisinfo.com/ and will not be reviewed in detail here.

Several methods can detect the increase in apoptosis. The first is the TUNEL assay (Terminal deoxynucleotide transferase dUTP Nick End Labeling), where the changes in nuclear morphology and staining characteristics were detected using a microscope. Another method combined TUNEL staining techniques with the flow cytometer to measure apoptotic cells. Again, the flow cytometer method made it possible to determine the stage of the cell cycle when the cells were undergoing programmed cell death. Finally, time-lapse photography has proved to be useful in evaluating the role of cell cycle, mitotic arrest, differentiation, mitotic catastrophe, mitotic death, and apoptosis in radiation-induced cell killing (Chu et al. 2002; Chu et al. 2004).

Using microbeams and other techniques, it was determined early in the Program that radiation induces apoptosis not only in cells hit by radiation, but also in bystander cells (Lyng et al. 2000; Belyakov et al. 2002; Lyng et al. 2002a, b). Early research focused on the signals and the critical genes and proteins involved in the induction of apoptosis. It was determined that different forms of the stress-inducible polypeptides called clusterin played a key role in radiation-induced apoptosis (Kalka et al. 2000; Leskov et al. 2001a; Leskov et al. 2003; Araki et al. 2005). Radiation-induced activation of critical genes and proteins including nuclear clusterin were found to play an important role in radiation-induced apoptosis (Yang et al. 2000b; Klokov et al. 2004). It was also determined that clusterin played a key role in signaling and acted as a molecular sensor between DNA damage and cytoplasmic responses (Huang et al. 2000; Davis et al. 2001). Thus, transcription factors activated by low doses of radiation resulted in apoptosis and were dependent on the p53 status of the cells (Criswell et al. 2003a; Criswell et al. 2003b). It was suggested that this cell killing in bystander cells could be selective against cells with genomic instability and transformed cells. Such differential cell killing was postulated to result in antitumor activity and a protective effect from low doses of radiation exposure (Boreham et al. 2000; Kagawa et al. 2001; Bassi et al. 2003; Bauer 2007b; Bauer 2007a).

3. Teratogenic effects

A biological major change observed in the A-bomb survivors was the development of birth defects in individuals exposed during fetal development. The relationship of these effects to low doses of radiation and the role of cell killing during the development of the embryo remains an important question.

The Program has funded a number of studies using fish embryos to address, at the cell and molecular level, the impact of low doses of radiation on embryonic development. In these studies, zebrafish were exposed to low levels of radiation during embryogenesis and the effects monitored. With this experimental system it was possible to irradiate different parts of the developing embryo, quantify the induction of cell death in situ, and determine the impact of killing cells in these different regions on the development of birth defects (Bladen et al. 2007b). Studies were also conducted at the molecular level to determine if there were biological responses that could protect these fish from exposure to low doses of radiation.

It was determined that increased expression of the subunit XRCC6 of the Ku70 proteins, which are involved in regulation of the cell cycles, protected the zebrafish against the development of birth defects (Bladen et al. 2007a). With further development, these systems have the potential to provide important basic mechanistic information on the role of radiation in the development of birth defects if humans were exposed in utero.

All these new techniques and biological systems have made it possible to address important questions in low dose radiation biology and to generate a large amount of data on the response of many biological systems to exposure to low doses of ionizing radiation. These observations are reviewed in Chapter 5, and many have resulted in new paradigms in radiation biology. The data from the Program research conducted using these techniques has also helped address many practical problems, such as developing new methods for biodosimetry, understanding low dose rate effects and evaluating the potential usefulness of some of the factors used in radiation protection, such as the DDREF and radiation weighting factors (w_D).

Major Points: Application of New Technology

The DOE Low Dose Radiation Research Program was essential for the development of microbeam and biological and molecular technology.

- Single-cell irradiation systems using alpha particles were developed at several locations
- An electron gun was developed to expose small numbers of cells to beta particles
- Equipment was developed to deliver focused X-rays to individual cells. These used a range of energies to be representative of the type of gamma rays that are present in the environment.
- Technology developed to differentially stain individual chromosomes was used by the Program along with cell sorting to characterize cytogenetic damage as a function of dose, dose-rate and radiation type.
- The use of cell and molecular techniques developed in the Human Genome Program made it possible to measure changes in radiation induced gene expression in large numbers of genes as a function of radiation dose. Some genes were turned on at low doses and different genes were turned on at high doses.
- New DNA repair technology using □H2AX foci made it possible to determine the number and location of nuclear traversals from microbeam irradiation.

- Techniques were developed that made it possible to detect multiple damage sites in DNA.
- Using creative assays DNA repair genes were identified and characterized.
- Methods to measure cell killing were improved to define the fine structure in the dose response relationships. This demonstrated non-linear responses in the low dose region.
- The identification of apoptosis was improved using modern technology. It became possible to identify selective cell killing of transformed cells through apoptosis.
- Zebra fish were exposed with the microbeam to irradiate different parts of the developing embryo and relate cell killing to birth defects.
- Early technique developments in proteomics, secretomics, and metabolomics were important in detecting metabolic biological changes as a function of radiation dose.

Chapter 5

Paradigm Shifts in Low Dose Radiation Biology and Application of Data

With the development of new tools and more sensitive techniques described in Chapter 4, it became possible to make several important observations and discover new phenomena describing how biological systems respond to low doses of radiation. The discovery and characterization of these new responses was an important early accomplishment of the DOE Low Dose Radiation Research Program. This chapter discusses these and shows how these discoveries made it necessary to change the traditional thinking in radiation biology and develop new paradigms on the response of biological systems to low doses of radiation. The major early discoveries were related to three unique biological responses: bystander effects, adaptive responses, and genomic instability.

Bystander effects are the observation that cells and tissues communicate with each other and that when radiation insults one cell it results in a response in the neighboring cells that have no radiation energy deposited in them. It has long been known that there is extensive cell-cell, cell-matrix, and cell-tissue communication, and the matrix and cell-cell interaction influence changes in gene expression ((Bissell & Aggeler 1987; Bissell & Barcelloshoff 1987). The functional units for cancer induction were thus shown to be units larger than cells, and it was suggested that it takes a tissue to make a cancer, not simply changes in the individual cell (Barcellos-Hoff & Brooks 2001).

With the development of microbeams and other techniques, this became obvious to the field of radiation biology. Early studies made this observation for many different biological systems and were able to relate such a response back to previous research that suggested that the “hit theory” for describing radiation response needed to be modified because the targets for biological response were much larger than individual cells (Brooks 2005).

In the early part of the Program, adaptive responses were described as “any responses to low doses of radiation that changed the magnitude and direction of the biological response to subsequent radiation exposure.” This term was later expanded to include the observation that low doses of radiation could also reduce the background level of biological alterations for a wide range of different biological systems.

In many different biological systems, adaptive responses were shown to have a marked influence on the shape of dose-response relationships in the low dose region. It decreased the magnitude of the response in the low dose region below that predicted from a linear extrapolation from the high dose region. Because of this decrease, the adaptive response has also been called a protective or a “hormetic” response to low doses of radiation. This phenomenon has been the center of many scientific discussions and arguments on the shape of the radiation-induced dose-response relationship for the induction of disease (Tubiana 2005; NRC 2006).

Radiation can alter the genomic stability of cell and tissues. Early research was reviewed and demonstrated that radiation-induced genomic instability was observed many cell divisions after radiation exposure. Genomic instability was manifested by an increase in the frequency and type of chromosome aberrations (Morgan et al. 1996). Soon after radiation, cells would divide and return to a "normal" state. After multiple cell divisions, genomic instability would develop, and many cells with chromosome abnormalities, most of which were not the result of clone formation, were observed in the population. Genomic instability was shown to be a frequent event per unit of radiation dose, so the target for its induction was much larger than a single gene. It was thus not related to a simple mutation in a single gene (Limoli et al. 1999; Ullrich 2003). However, it was difficult to demonstrate the changes in the frequency of genomic instability following low doses of radiation.

(Kadhim et al. 1995) observed radiation-induced genomic instability in bone marrow cells from both humans and rodents. (Ponnaiya et al. 1997a) linked genomic instability to the sensitivity of different strains of mice to the induction of breast cancer, suggesting that it is an important step in radiation-induced cancer The observation of genomic instability has made it necessary to alter paradigms associated with the influence of single mutations on the induction of cancer and to take a more holistic view suggesting that tissue responses to genomic instability may be an important part of radiation-related cancer.

It was also demonstrated that the genetic background of the cells/tissues and organisms was very important in the magnitude and frequency of each of these new phenomena.

I. Bystander Effects: Cell-Cell and Cell-Tissue Communication

Before the Program, cell-cell and cell-matrix communication and interactions were recognized as important in altering biological responses to many environmental insults. Such interactions play an important role on malignant phenotype during radiation-induced cancer (Bissell & Barcelloshoff 1987; Trosko et al. 1990; Park et al. 2000).

(Nagasawa & Little 1992) published one of the earliest reports demonstrating that the target for the effects of ionizing radiation was larger than the cell nucleus. They observed that when CHO cells were exposed to a collimated alpha source at very low doses (0.31 mGy), 30% of the cells had an increased frequency of sister chromatid exchanges (SCE), even though fewer than 1% of the cells were calculated to have been traversed by an alpha particle.

Additional studies confirmed that many more cells were responding with an increased frequency of SCEs than had energy deposited in them (Lehnert & Goodwin 1997). This observation was in direct conflict with the current target theory and resulted in major discussions. The importance of bystander effects in the induction of SCE on cancer risk was questioned. There was concern that the observed increase in the frequency of SCEs as a bystander effect may not impact radiation risk (Bonassi et al. 2004). In addition, at this time it was impossible to tell which cells had energy deposited in them and which affected cells were neighbors, or bystanders. It was only known that fewer cells were hit than were responding.

The microbeam made it possible to place the alpha particles in known cells so that the cells that were hit and had energy deposited in them could be identified. This enabled biological changes to be studied in cells with and without energy deposited in them (Prise et al. 1998; Prise et al. 2002; Braby et al. 2006). After alpha particle microbeams were developed, additional equipment was developed that made it possible to use focused X-rays (Prise et al. 2003) and beta particles (Sowa et al. 2005; Persaud et al. 2007) to place energy in known cells and study the biological responses of both the hit cells and the bystanders. These studies suggested that bystander effects such as cell killing and micronuclei could also be seen following the exposure of individual cells to low-LET radiation (Prise et al. 2003; Schettino et al. 2003; Resat & Morgan 2004b; Persaud et al. 2007). All the cells could then be evaluated. It was determined that many cells were responding with biological changes without the deposition of energy.

The use of these tools and other techniques on both hit cells and non-hit cells in the same culture dish, separated with medium such that there could be no energy deposited in one set of cells while the others were being hit (Geard et al. 2002), or using the media from hit cells to initiate responses in cells that were not exposed to radiation, resulted in several publications. (Prise et al. 1998; Lyng et al. 2000; Azzam et al. 2002; Suzuki et al. 2004; Yang et al. 2005).

From these studies, it became evident that there are two basic types of bystander effects. First, there is direct cell-cell and cell-matrix communication that requires that the cells to be in direct contact with each other (Azzam et al. 1998; Azzam et al. 2001; Nagasawa et al. 2002; Azzam et al. 2003a, b; Mitchell et al. 2004b) or with the matrix (Barcellos-Hoff & Ravani 2000; Park et al. 2000; Barcellos-Hoff & Brooks 2001). This contact is dependent on the presence of gap junctions between the cells and can be blocked by substances that inhabit gap junction function (Azzam et al. 2001, 2003a, b).

The second type of bystander response demonstrates that cells with energy deposited in them release soluble factors, hormones, cytokines, or clastogenic factors into the media or the tissues. These produce alterations in other cells that do not have energy deposited directly in them (Lyng et al. 2000; Mothersill & Seymour 2001; Suzuki et al. 2004; Mothersill et al. 2005; Yang et al. 2005). These “media transfer” studies were conducted for a wide range of different systems where the media from irradiated cell (both following high- and low-LET exposure) were transferred to non-irradiated cells and biological effects measured and demonstrated in the non-exposed cells.

It is important to recognize the wide range of biological endpoints that are modified by bystander effects. The earliest research on bystander effects using the microbeam was conducted in monolayer tissue cultures *in vitro.* Cell killing through apoptosis was an early endpoint that could be easily measured both in cells that had energy deposited in them and in their neighbors (Prise et al. 1998; Belyakov et al. 2001; Prise et al. 2002). Elimination of cells by apoptosis can potentially result in a decrease in damaged or transformed cells from tissues or organs and could result in a protective effect. These effects will be discussed in more detail in the section on adaptive responses later in this chapter.

Early in the Program, research was conducted on the ability of alpha particles to cause cell transformation. The tissue culture cells used in transformation studies were already altered and

well on the way to the development of cancer. The transformation endpoint measured is an indication that radiation can move the cells through the final steps needed to move normal cells to cancer cells.

These early studies demonstrated that cell transformation *in vitro* could be induced by a single alpha particle (Miller et al. 1999). In tissue cultures where every cell had one alpha particle deposited in it, the transformation frequency was lower than in cultures where the alpha particles were randomly distributed with an average of one alpha particle per cell. It was postulated at the time that perhaps more than one alpha particle was necessary to induce cell transformation. As research has continued, it became obvious that many of the transformed cells may have been bystanders with no energy deposited in them. By conducting studies where only a small fraction of the cells in the population had alpha particles deposited in them, it was determined that in cells that did not have energy deposition in them, bystanders could also be transformed (Sawant et al. 2001b; Mitchell et al. 2004c). This transformation was not dependent on the number of alpha particles deposited in the cells or the fraction of the cells exposed. Thus, these studies demonstrated that bystander cells that did not receive any energy deposition could be transformed by direct communication from exposed cells through those final stages from normal to cancer.

The kinetics of the initiation of cell transformation demonstrated that there was an early rise in transformation frequency with exposure to a small number of cells to single alpha particles, and that the frequency of transformation remained rather constant as a function of the number of cells exposed or the number of alpha particles that traversed the cells (Sawant et al. 2001a). Figure 9 demonstrates these “on/off” or “all or none” non-linear kinetics for the induction of cell transformation and mincronuclie in bystander cells, which have been demonstrated in a number of cell systems and for a variety of endpoints including the induction of micronuclei (Belyakov et al. 2001; Azzam et al. 2002; Ponnaiya et al. 2004).

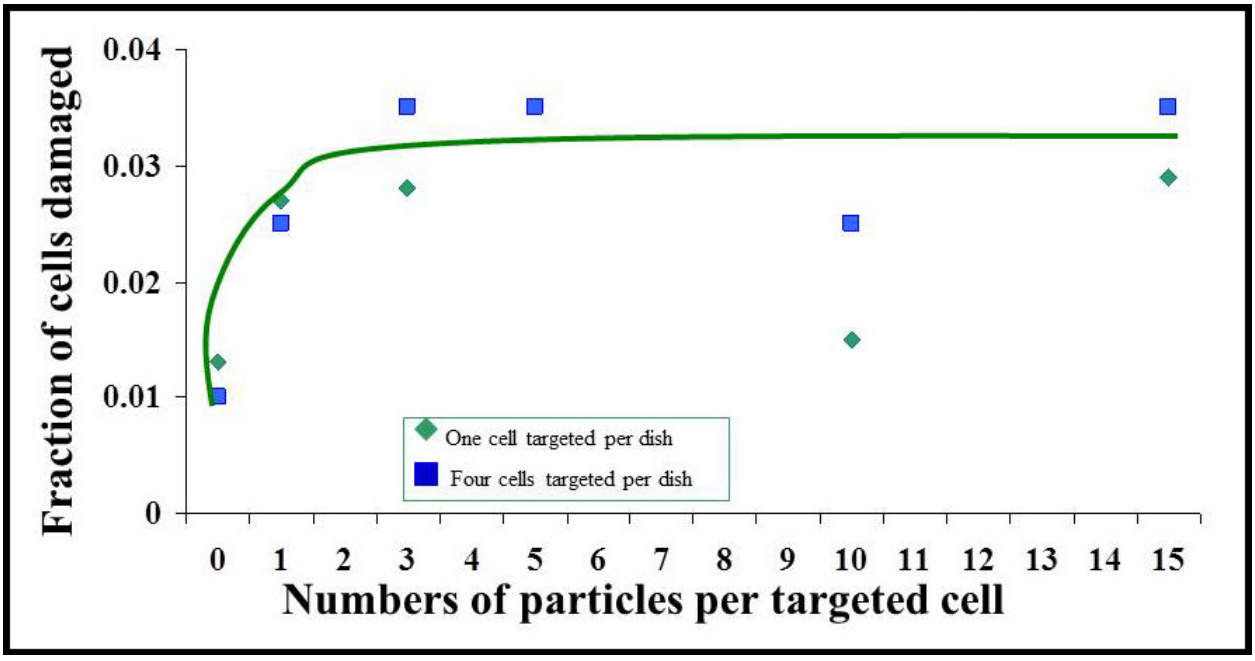


Figure 9. Bystander effect showing off-or-on type of dose response. Increasing the number of alpha particles per hit cell did not modify the frequency of micronuclei in non-hit cells (Bennett et al. 2007).

The slope of the hit-response relationship for the induction of micronuclei in cells that had alpha particles deposited in them was similar to that reported in other studies (Nelson et al. 1996; NRC 2005). However, in the bystander non-hit cells there was a non-dose-dependent increase in the number of micronuclei above that observed in control cells (~2-fold). These types of binary behavior in the dose-response kinetics were also demonstrated using low-LET microbeams(Schettino et al. 2005). These dose-response relationships have been evaluated and many models developed to predict the impact of bystander cells on risk. These models will be further evaluated in Chapter 7.

Because of the ease of scoring, the frequency of micronuclei has been used as an endpoint to evaluate the role of many experimental conditions on the induction of bystander effects. Bystander effects have been studied under a wide range of experimental conditions using micronuclei as an endpoint. Research demonstrated that the target for induction of bystander effects was the nucleus. Cells were radiated with microbeams under conditions where only the nucleus and cytoplasm had energy deposited in them (Shao et al. 2004), and bystander effects were observed only when the nucleus was "hit". The influence of genetic background of the system studied had a marked influence on bystander effects (Zhou et al. 2005). It was determined that intercellular communication (Azzam et al. 1998; Azzam et al. 2001; Azzam et al. 2002; Shao et al. 2003a) was essential for the induction of bystander effects. The oxidative status of the cells altered the frequency of micronuclei and bystander effects (Azzam et al. 2002). It was determined that cells in different stages of the cell cycle had marked influence on the observation of bystander effects (Balajee et al. 2004), and the influence of time and distance between the cells with energy deposited in them and the bystander cells (Belyakov et al. 2002). (Wu et al. 2006) also determined that as the energy across the Bragg peak changes there is little change in the frequency on micronuclei as a function of energy deposited in a localized area. This again suggests that bystander effects are acting across this system to result in similar responses.

The types of cell and molecular-level damage observed in cells with direct passage of alpha particles was demonstrated to be different than that in bystander cells. In cells with direct energy deposition, scientists observed that the majority of the mutations were of the deletion and loss type, the same as was previously demonstrated for the induction of mutations from ionizing radiation (Jostes et al. 1994; Schwartz et al. 1994). However, most of the mutations induced in bystander cells were point mutations, base substitutions, and base changes that are more closely related to spontaneous mutations observed in control cells and mutations induced by chemicals (Jostes et al. 1994; Schwartz et al. 1994; Huo et al. 2001; Zhou et al. 2003). This important difference in mutation types could markedly affect the impact on radiation risk. However, the radiation risk associated with the induction of genetic disease has been evaluated, and it has been determined that the risk for genetic disease is 0.4 to 0.6% per gray of the very high baseline frequency (738,000 per million) (NAS/NRC 2006, Phase 2, pages 91-131). This low value illustrates that the risk for genetic disease is much lower than the risk for induction of cancer,

about 10% per Gy against a background frequency of cancer of 40%, and is not the primary concern when setting radiation standards (NAS/NRC 2006, Phase 2, pages 91-131).

Studies on the induction of chromosome aberrations in bystander cells also demonstrated a marked difference in the type and frequency of chromosome aberrations induced in cells that were directly hit with ionizing radiation and those induced in bystander cells. It was again observed that the slope of the linear dose-response relationships for the induction of chromosome aberrations and micronuclei by alpha particles was similar to that previously reported (Brooks et al. 1994; Miller et al. 1996; Nelson et al. 1996; 1999; Geard et al. 2002).

In bystander studies, using the technique where cells that were exposed were separated by medium in a way that one side of the flask received direct energy deposition in the cells while the thickness of the medium was such that the cells grown on the other surface were out of the range of the alpha particles and had no energy deposited in them (Geard et al. 2002; Suzuki et al. 2004). The aberrations in cells with or without energy deposition were scored using the premature chromosome condensation (PCC) technique. It was discovered that cells with direct energy deposited in them contained chromosome-type aberrations (Suzuki et al. 2004). Again there was a non-linear dose-response relationship in the bystander cells, with a marked increase in bystander cell aberrations, above that seen in the control cells. The other important observation in bystander cells was that most of the aberrations were of the chromatid type. These types of aberrations are not normally produced by radiation of cells that are in the G_1 stage of the cell cycle. This indicates that the chromatin must have been damaged in the bystander cells to produce these aberrations. Chromatid-type aberrations are also a hallmark of cells that are becoming genomically unstable (Kadhim et al. 1995).

As studies have expanded, it has become increasingly evident that many biological changes are induced in bystander cells. An important observation has been that cells that do not have energy deposited in them have a change in gene expression following irradiation of cells that are communicating with them (Azzam et al. 1998). Changes in gene expression have been very useful in understanding both bystander effects and other low dose radiation effects.
In addition to the formation of γH2AX in cells directly traversed by alpha particles (Prise et al. 2002), it has also been demonstrated that bystander cells have increased frequency of γH2AX and DNA DSBs (Sokolov et al. 2005; Smilenov et al. 2006). During repair (Little 2003) these DSBs can result in the formation chromosome aberrations and γH2AX focus. This is expected because bystander cells also induce chromosome damage resulting in both chromatid aberrations and micronuclei.

The biology of bystander cells is modified in many ways. Studies have demonstrated that bystander cells have modifications in the cell cycle (Balajee et al. 2004), and there is evidence for chromatin damage in bystander cells that results in the induction of chromatid-type aberrations (Suzuki et al. 2004). In organized tissues, bystander cells seem to be forced to differentiate in non-standard ways (Belyakov et al. 2002, 2006).

It is important to understand the physical and biological variables associated with the bystander response. A number of important scientific questions relative to the bystander effects are posed,

and the early research addressing these questions will be examined. To fully understand the significance of bystander effects to cancer risk, the following questions need to be answered.

- What is the cellular target for the initiation of the bystander response?
- Which cell types can communicate with each other?
- Can all the cells in a tissue respond to bystander signals?
- What molecules and structures are involved in communication of the bystander effects?
- What is the time required for the communication?
- Over what distance can cell-cell communication be observed for the cell-cell contact type of bystander effect?
- Do the bystander effects occur in whole organisms within tissues and between different tissues?

Many studies using microbeams have demonstrated that the prime target for the induction of bystander cells is from deposition of energy in the nucleus of the cell using either high- or low-LET radiation (Prise et al. 2002; Morgan 2003b; Schettino et al. 2003; Zhou et al. 2003; Hall 2006). Exposure of only the cytoplasm has also been shown to initiate bystander effects (Shao et al. 2004), but the response is not as robust as deposition of energy in the nucleus.

It has been noted that the communication between cells is almost universal when the cells are the same type. Thus, studies demonstrated that fibroblasts, epithelial cells, and other cells can communicate with each cell type. However, it has also been demonstrated that only a limited number of individual cells within any tissue can respond to a bystander signal, and that material from one cell type can stimulate a bystander response in other cell types. These studies have been carefully reviewed (Lorimore et al. 2003; Morgan & Sowa 2005; Hall 2006). Nevertheless, it has been demonstrated that communication between some different cell types can be limited. For example, fibroblasts and glioma cells communicate within each cell type, but communication between the different cell types appears to be unidirectional (Shao et al. 2004). The fibroblasts can communicate and produce responses in glioma cells, but exposure of the glioma cells does not seem to be able to produce changes in the co-cultured fibroblasts.

Extensive research has been conducted to determine how the signals are transmitted from one cell to the next during the initiation of the bystander response. It has been well established that the membranes between the cells play an active role in the communication of the messages between cells (Azzam et al. 2001; Nagasawa et al. 2002) and that the extracellular matrix also plays an important role in signaling and controlling the fate of cells (Barcellos-Hoff & Brooks 2001).

The physiological state of cells plays an important role in bystander communication. For example, it has been established that inflammatory responses play an important role in cell-cell communication (Lorimore et al. 2003). The redox status of the cells (Spitz et al. 2004; Hu et al. 2006), the energy and oxidative metabolism (Mothersill et al. 2000; Azzam et al. 2002), and oxidative stress related pathways responses and the molecules associated with them are all involved in bystander effects (Azzam et al. 2002; Spitz et al. 2004; Hu et al. 2006). The nutritional status of the cells and cell/cell contact are important in bystander responses and in the

production of radiation induced hypersensitivity(Chandna et al. 2002). Studies are continuing on the molecules involved. The molecules and mechanisms involved in the communication are described in Chapter 6.

The length of time required for an exposed cell to produce a response in a bystander cell has also been the subject of research and has been reviewed (Morgan 2003b). For direct cell-cell communication the length of time required for transmission of signals is very short (<1 minute), so that direct cell-cell communication is involved (Little et al. 2002a; Banaz-Yasar et al. 2006). After stimulus of cells by radiation, substances are secreted into the medium very quickly after the exposure that are responsible for bystander effects.

The distance over which bystander effects can be seen has been an important area of research. Using culture systems it was evident that the bystander cells can be detected throughout the whole culture dish (Azzam et al. 1998; Prise et al. 1998; Azzam et al. 2001; Prise et al. 2002) (Belyakov et al. 2001; Sawant et al. 2001b; Ponnaiya et al. 2004) and that the distribution of the cells displaying bystander effects seems to be randomly distributed over the dish (Azzam et al. 2001; Prise et al. 2002). These data have been reviewed (Morgan 2003b) and suggest that all the cells on the dish are in communication with each other.

When more specialized three-dimensional cell systems were devised *in vitro,* a range of different results were observed. In a system where a strip of cells was exposed using a specialized microbeam, the communication was limited to a few cells in close contact with the exposed cells (Belyakov et al. 2006). In this system the cell-cell communication was only a matter of a few cell diameters. More complex tissue systems were developed using both human and porcine urothelial explants where it was possible to detect a wide range of different cell responses under more realistic physiological conditions (Belyakov et al. 2002, 2003). This system was exploited to detect bystander changes as a function of distance from the exposure and is illustrated in Figure 10.

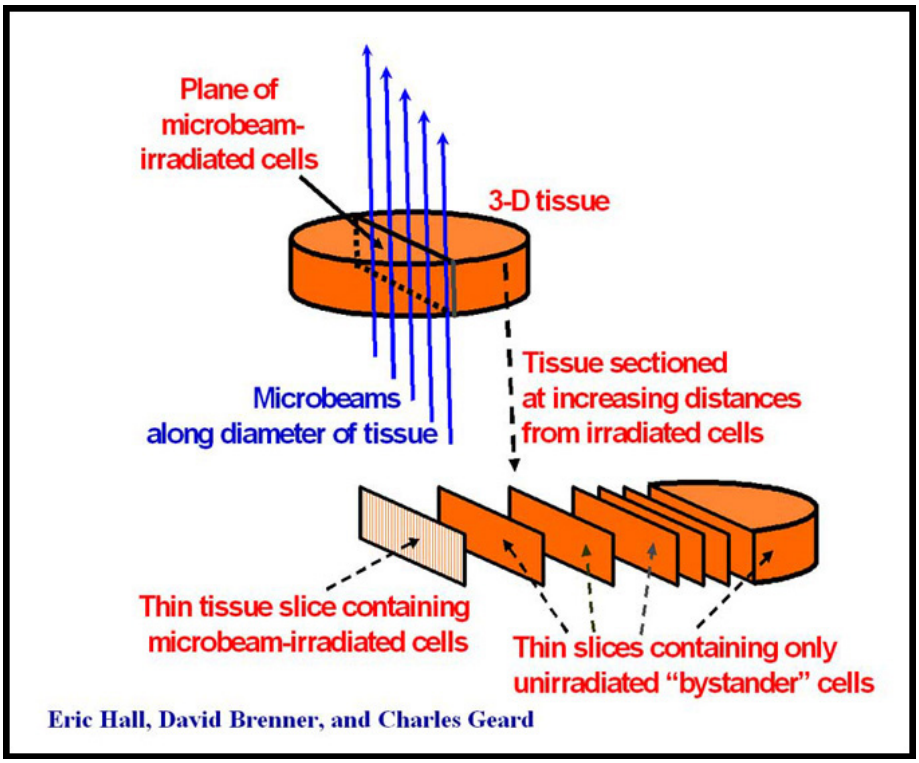


Figure 10. A plane of cells was irradiated using a microbeam. Bystander response was measured in cells at different distances and times from the hit cells, making it possible to see how fast and far from the hit cells the bystander response could be detected (Belyakov et al. 2005).

The tissue was exposed at a known location, and sections were taken as a function of distance from the exposure. The frequency of apoptotic cells (Belyakov et al. 2002) and cells undergoing premature differentiation (Belyakov et al. 2006) were measured in each section. It was determined that a constant frequency of altered bystander cells could be detected as a function of distance from the target. These changes were detected over a range of up to 1 millimeter from the radiation site (Belyakov et al. 2005). This system was also very useful for measuring bystander induced cell proliferation (Belyakov et al. 2003).

Such measurements suggested that bystander effects could be detected in whole human or animal tissues. The existence of clastogenic factors that cause chromosome damage when cells are treated with serum from radiation-exposed animals have long been recognized and have been reviewed (Morgan 2003a). When one part of an organ, the lung, was exposed to high doses of radiation, responses (micronuclei formation) were seen to be greatly elevated in the remainder of the lung. It seems that certain lobes of the lung communicate the existence of damage in a directional fashion, from the bottom to the top. These responses were limited to the lung (Khan et al. 1998).

Other evidence for bystander effects *in vivo* was reviewed and includes both the formation of chromosome aberrations and cancer in the liver (Brooks 2004). In these studies, the frequency of both chromosome aberrations and lung cancer was the same whether 1% or 100% of the liver

cells had energy deposited in them from internally deposited $^{239}PuO_2$ particles. The frequency of change in both endpoints was dependent on the total dose to the organ and not on the number of cells hit or the dose to individual cells. However, when animals were exposed to internally deposited radioactive materials, which concentrated in target organs, it was noted that the bystander effects were not evident. Only the organs with the isotopes deposited in them resulted in the formation of cancer (Stannard et al. 1988).

It has been postulated that a small amount of radiation over time, such as the low dose rates from these exposures, results in a low level of clastogenic or any other soluble factors. It has been noted that following high dose-rate exposure to high total doses, soluble compounds seem to result in either the cause or cure of cancers at sites distant from the radiation. The role of ascopal effects, bystander effects, and clastogenic factors in the induction of genomic instability and cancer was reviewed (Morgan 2003a).

A number of reviews have been written on bystander effects and provide extensive additional information on these effects (Hall 2000b; Mothersill & Seymour 2001; Hall 2003; Morgan 2003b, a; Azzam & Little 2004; Hall 2006). These reviews presented conclusive data demonstrating that the cells did not need to have energy deposited in them to elicit a wide variety of biological responses. It seems that many small molecules including calcium (Lyng et al. 2006) and nitric oxide molecules may be involved (Shao et al. 2003b) in transmission of the bystander responses between cells. Additional discussion on the pathways, such as the MAP kinase signaling pathway (Lyng et al. 2006) and other mechanistic studies, is found in Chapter 6.

The observation of bystander effects in so many molecular, cellular, and whole animal studies impact the use of hit theory to understand the relationships that exist between radiation dose and biological responses. The use of hit theory must be modified in light of the bystander effects to show that the target size for the biological response is much larger than the cell nucleus.

Cell transformation and other biological changes in the DNA are important endpoints that suggest that bystander effects could increase the risk at low radiation doses (Hall 2000a). The importance of these responses in terms of risk assessment remains an open question and has been reviewed (Morgan & Sowa 2009). However, a recent publication suggested that they were not able to detect bystander effects following exposure to high-LET radiation (Groesser et al. 2008). These studies measured several crykogenetic endpoints including the induction of micronuclei, γH2AX, and cell killing. Additional studies need to be conducted to help resolve this observation with the literature that has been published on the induction of bystander effects by high-LET radiation using several experimental systems.

There have been a number of publications on the impact of bystander effects on genomic instability and the risk for the induction of cancer (Brenner & Elliston 2001; Brenner & Sachs 2002a, 2003; Morgan & Sowa 2009). From these studies it was concluded that the bystander effects of alpha particles may influence the shape of the dose-response curve but that the risk that is currently used to estimate radiation risk, for example for radon, may not be markedly influenced by the bystander effects (Brenner & Sachs 2003). Additional discussion of the risks associated with bystander effects is covered in Chapters 7 and 8.

Major Points: Bystander effects

- Cells that have energy deposited in them communicate with neighboring cells, which do not. These bystander effects exist both in vitro and in vivo.
- There are two different types of bystander effects; those that require direct cell-cell and cell/matrix contact and those that result from release of substances into the media or blood.
- The bystander effect results in changes in several different biological endpoints and is dependent on the physiological and oxidative status of the cells and tissues.
- The dose-response relationships for the induction of bystander effects are non-linear with a low-dose resulting in the maximum response followed by a plateau as the dose increases.
- The type of damage in bystander cells is different from the type of damage induced in cells with energy deposited in them.
- Because of bystander effects, tissues respond as a whole to ionizing radiation and not as single cells. These tissue responses are non-linear.
- There is evidence that bystander cells may either increase or decrease the radiation related cancer risk.

II. Adaptive Responses

Many physical and chemical agents are toxic when given at high doses (e.g., vitamins, aspirin, many toxic agents, stress) but have protective and beneficial effects when given at low doses (Luckey 1991; Calabrese 2004). For example, vitamins are very toxic at high doses but are essential for life at low doses. Exercise also has beneficial health effects even though it generates many free radicals that are known to be damaging and increase cancer risk when levels are too high. These protective non-linear dose-response relationships between exposure, dose, and response have been extensively reviewed and are collectively termed hormesis (Calabrese & Baldwin 2003). Hormesis is the production of a beneficial effect caused by a low dose of an insult.

For radiation, it has been long assumed that each ionization has the potential to produce DNA damage, and that DNA damage is linearly linked to the formation of adverse health effects including cancer and genetic effects. Thus, models have been developed that predict that damage and risk from radiation exposures increase linearly with radiation dose. Data support this idea for the induction of DNA damage. However, recent research, much of which was funded by the DOE Low Dose Radiation Research Program, has demonstrated that the processing of radiation-induced damage and the total response to radiation in the low dose region is non-linear. This non-linear processing can result in “protective adaptive responses” in the low dose region. Many biological responses to radiation are very different in the low dose region from those seen in the high dose region. Such research predicts that the mechanisms of action for biological responses change as a function of radiation dose.

The prime argument against a protective adaptive response for radiation has involved the way that radiation interacts with and alters cells. Radiation deposits its energy in discrete sites in cells and molecules. The biological action on these sites is randomly distributed in the tissue and is unique. Because of these facts, it was suggested that radiation-induced damage cannot be compared to damage from chemicals or stress, where all molecules and cells in a tissue organ or organism will receive the exposure uniformly and have the potential for being affected. However, extensive research reviewed in the last section of this chapter illustrates the role of bystander effects following radiation exposure. These effects demonstrate that even though the energy is deposited in random defined sites and the initial DNA damage increases as a linear function of exposure, radiation effects are not limited to the individual cells where the energy is deposited. The whole biological system responds to the insult in the same way as seen for chemicals, and the processing of the radiation damage is non-linear. This non-linear processing of radiation-induced change supports the existence of protective adaptive responses.

Because of these observations and the biological complexity associated with cancer induction, a systems approach rather than the use of the hit theory to predict radiation effects is required and needs to be the focus of future research. Thus, both scientific opinion (Jenkins-Smith et al. 2009) and research reviewed here currently support the existence of non-linear dose-response relationships in the low dose region.

The field of radiation-induced hormesis has been carefully reviewed and several literature citations gathered to support the concept that low doses of radiation can have a protective effect for many different endpoints (Calabrese & Baldwin 2003). From this research it is suggested that in the low dose region hormesis predicts the response to many agents better than other models, including the LNT and threshold models (Calabrese et al. 2007). The hormetic, or potential protective effect, from low doses of ionizing radiation continues to be debated, and the impact of low doses on radiation risk is a major subject of controversy (Brenner et al. 2003; Feinendegen 2005; NRC 2006; Averbeck 2009; Brenner 2009).

The diminished response of a biological system to low doses of radiation has been termed “adaptive response.” Adaptive response was first demonstrated in studies where cells were treated with tritiated thymidine followed by exposure to large doses of X-rays (Olivieri et al. 1984). Surprisingly, the frequency of chromosome aberrations with the tritiated thymidine followed by the high dose of X-rays was lower than when the X-rays were given alone. These studies were followed up by exposing cells to a priming dose, very low doses of X-rays (10-50 mGy), followed soon after by a larger (1.0-2.0 Gy) challenge dose. The frequency of chromosome aberrations induced by the challenge dose when the cells had received a prior small or “tickle” dose was reduced relative to that observed when the challenge dose was given alone. This observation is illustrated in Figure 11.

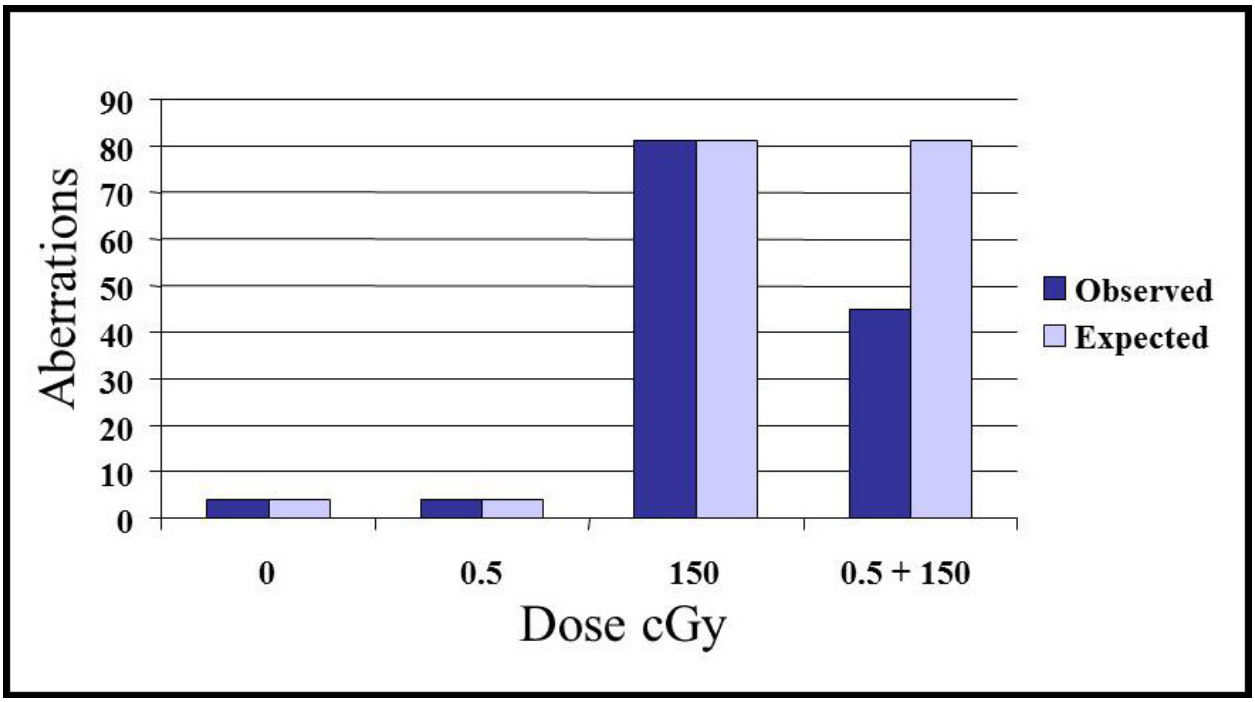


Figure 11. Expected and observed results from a small radiation dose followed by a larger dose showing that a small dose decreases the response, indicating the presence of an adaptive response (Shadley et al. 1987).

Many publications resulted on the reduction in the frequency of chromosome aberrations as an indication of the induction of adaptive responses. The results of these studies were carefully reviewed by (Wolff 1998), who noted that that the genetic background of the biological materials used in the test plays an important role in the adaptive response. For the people tested for adaptive responses, many individuals were "responders," and others were not. The importance of genetic background on the induction of bystander effects, adaptive responses, and genomic stability will be discussed further as it applies to each of these biological observations following low doses of radiation. The classic "adaptive response" has been demonstrated for several different biological endpoints including the induction of apoptosis (Boothman et al. 1998), cell killing (Sahijdak et al. 1994; Mitchell & Joiner 2002), micronuclei (Shankar et al. 2006), cell cycle changes (Boothman et al. 1996), gene expression (Coleman et al. 2005), mutations (Zhou et al. 2003; Sykes et al. 2006a; Tsai et al. 2006), and cell transformation (Redpath et al. 1987; Azzam et al. 1994).

An important system for measuring the adaptive response *in vivo* was to measure the frequency of recombinational events using the pKZi recombinational mutation assay in mice (Sykes et al. 2006a). This test could measure the impact of low doses of ionizing radiation in depressing the response induced by a high dose of radiation. In many of the research projects conducted, the level of mutation found was depressed below the spontaneous mutation frequency. With this experimental approach, there was a very complicated dose-response relationship between the exposure to very low doses as a priming dose and the frequency of mutations induced by the challenge dose. Following exposure to high doses (1 Gy) given alone, the frequency of mutations

showed a marked increase above the spontaneous level. Low doses given before the high dose resulted in a decrease in the mutation frequency below that observed in the control animals. As the tickle doses continued to decrease there was an increase in the mutation frequency.

There was some concern that this system was unique to the blood lymphocytes in which it was first measured. The argument was that the cells used in the assay were a part of the immune system and that the observed adaptive response was a reflection of the spontaneous rearrangements known to be made as adaptive responses of these cells to antigens. Thus, it was suggested this adaptive response might not be related to radiation-induced cancer.

To evaluate this potential concern, other tissues in the mice, such as the prostate (Hooker et al. 2004a) and spleen (Day et al. 2007a), were measured. It was determined that other somatic tissues showed the same decrease in the frequency of mutations, below that observed in the tissues of non-exposed animals, as was seen in the cells associated with the immune system. These studies resulted in very interesting dose-response relationships that require additional study. They demonstrated that genetic background of the mice had a marked role on the induction of this adaptive response (Hooker et al. 2004b).

Additionally, this system is the only one that has been able to demonstrate that the order of delivery of the doses, large challenge and small tickle, is not critical and that adaptive responses can be generated with both exposure schedules (Day et al. 2007a; Day et al. 2007b). The scientific concern about the importance of the classic adaptive response that induced by a small priming dose of radiation and then followed by a large challenge dose, in terms of radiation-induced cancer risk is that this type of "adaptive response" induced by the priming dose was only active for a short period of time. Thus, it was thought that the classic adaptive responses might have little impact on radiation risk.

In addition to the classical adaptive response, studies have been conducted on the influence of low doses of ionizing radiation on the background frequency of biological changes. This type of adaptive response may be more important in terms of the impact of low doses of radiation on cancer risk because it suggests low doses can be protective against many biological changes induced by other types of exposure as well as from the genetic background that may be involved in cancer induction. To conduct these types of studies, it was necessary for the endpoint of interest to have a rather high background rate, such as is seen for cell transformation and cancer.

A number of systems were developed to measure the influence of low doses of radiation on the spontaneous frequency of biological alterations and changes related to cancer induction. The most widely used of these systems measured the frequency of cell transformation as an endpoint. The primary cell transformation systems used were either a human hybrid cell system that has a high spontaneous frequency of cell transformation (Redpath et al. 1987) or the mouse embryo C3H 10T1/2 cell system (Azzam et al. 1994; Mitchel et al. 1997). With these tools, it was possible to expose the cells to low doses of radiation and determine the change in the frequency of cell transformation as a function of different dose parameters.

Many studies were conducted measuring cell transformation that showed low doses of ionizing radiation decreased the spontaneous frequency of cell transformation below that observed in

control cells receiving no radiation exposure (Azzam et al. 1994; Azzam et al. 1996; Redpath 2004, 2006a, b). An example of the type of results demonstrated in many of these studies is shown in Figure 12.

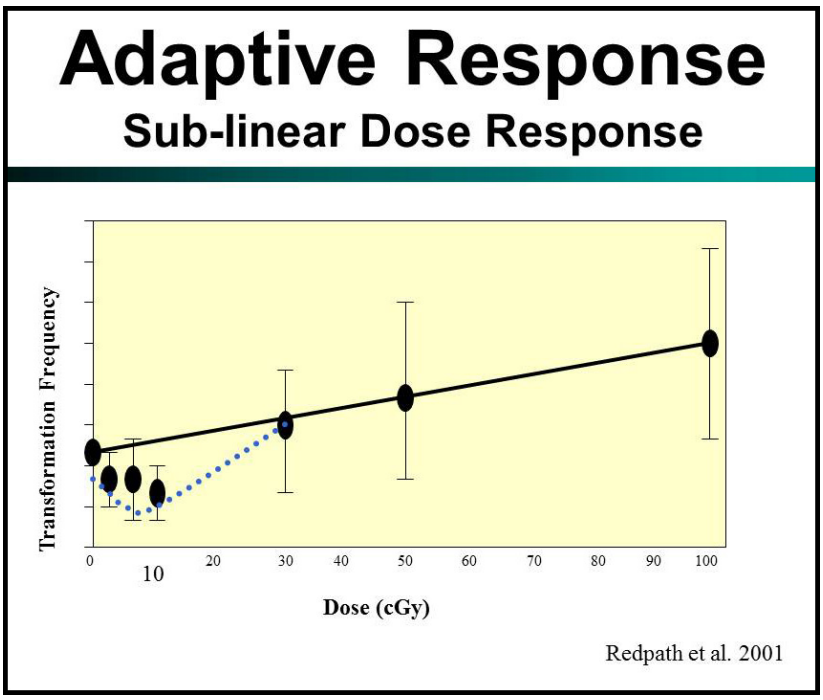


Figure 12. Sub-linear dose response demonstrating protective adaption where the low doses result in less damage than seen in the background response (Redpath et al. 2001).

Extensive studies have been conducted to determine the role of exposure variables on the induction of adaptive responses that decrease the spontaneous frequency of cell transformation. It was important to determine the role of total dose (Redpath 2006a, b) and dose rate (Elmore et al. 2006; Elmore et al. 2008) on the induction of adaptive responses. Figure 11 shows the results of low dose exposures (10-100m Gy) in decreasing the background rate of cell transformation. In the dose region below 100 mGy the frequency of cell transformation was decreased below that observed in the control non-exposed cells (Redpath 2006b). Thus, low total dose can reduce the frequency of transformed cells and may be protective.

When the dose rate was decreased, the response in the exposed cells was dependent on both the total dose and the dose rate. If the dose rate was delivered at 1.9 mGy/min there was a positive dose-response relationship for all dose groups all the way up to 1000 mGy. However, as the dose rate was decreased to 0.47 Gy/min and below, the response in the control cultures was higher than that observed in the groups exposed to ionizing radiation at all the doses evaluated, up to 1000 mGy. Thus, low dose-rate exposure may have a protective effect over a much broader total dose range than observed for single acute exposure.

It was also determined that cells exposed to chronic low dose-rate exposure had a higher survival rate when challenged with a subsequent acute radiation exposure than cells that had the challenge

dose only (Mitchell & Joiner 2002). Thus, low dose-rate exposure seemed to be protective for cell survival as well as cell transformation. When radiation dose was fractionated there was also a decrease in cell transformation (Bennett et al. 2007). It was important to determine if the types of radiation used in diagnostic procedures would induce such a response, since it has been predicted that the use of diagnostic radiation procedures, especially CT scans, could potentially result in a marked increase in total cancers in the population (Brenner & Elliston 2004). Research was conducted on the influence of diagnostic energy X-rays, (Redpath et al. 2003), and on mammographic energy X-rays (Ko et al. 2004). Both of these types of exposures resulted in a decrease in the frequency of cell transformation in the dose range used for diagnostic evaluations. The results of these types of studies were reviewed, and it was concluded that the low photon energies used in medical imaging all produced a reduction in the frequency of cell transformation (Redpath 2006a).

Additional studies on adaptive responses were conducted to simulate exposures found in the space environment. In these studies, low doses of proton exposure were given prior to exposure to HZE particles. This resulted in an adaptive response in a cell system using anchorage-independent growth in primary human fibroblasts as an endpoint, another measure of cell transformation (Zhou et al. 2006). The role of radiation type and changing LET on the induction of adaptive responses and bystander effects has been carefully reviewed (de Toledo et al. 2006). In this review, an adaptive response was observed over a wide range of LETs. Such a manuscript provides a useful reference for further study of these exposure variables on biological responses.

In addition to exposure variables, there were several molecular and cellular variables that play an important role in the observed adaptive responses. As it became possible to rapidly and accurately measure changes in gene expression, a number of studies were initiated to determine the role of low doses of radiation on changes in gene expression. Early in the Program these studies found that low doses of radiation could cause changes gene expression in many genes (Yin et al. 2003). The doses used in these studies were lower than those required to elicit a response using other cellular and molecular endpoints. This study was one of the best documentations that cells could detect low levels of radiation and respond to such low doses. As additional studies were conducted, it became obvious that the number and types of genes that changed expression were dependent on the radiation dose and dose rate (Coleman & Wyrobek 2006). This change in gene type as a function of dose can be seen in Figure 13.

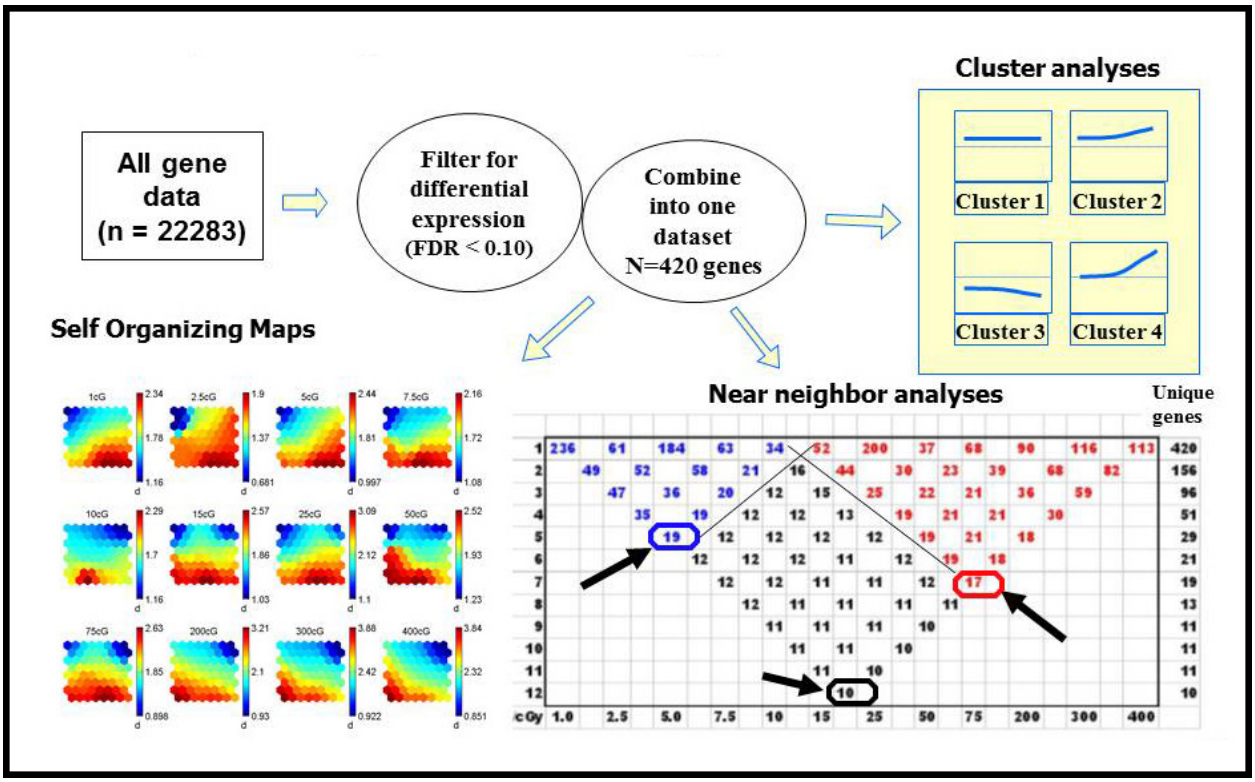


Figure 13. The dose-response relationship for gene expression of 22,283 genes. Self-organizing maps, near-neighbor analyses, and cluster analyses provide three lines of evidence pointing to a transition in transcript expression profiles as genes are up- and down-regulated in the 10- to 25-cGy range (Coleman & Wyrobek 2006).

Using three different techniques, 1) self-organizing maps, 2) cluster analyses, and 3) nearest neighbor analyses, the authors were able to demonstrate that there was a transition in gene transcript expression profiles in the range of dose from 0.1-0.25 Gy. This resulted in an important set of data that showed that cells recognize and respond to radiation as a function of radiation dose. Such data also suggest that the mechanisms of action in responding to radiation are different for low doses of radiation compared to those following high radiation doses.

The next task at hand was to link the changes in gene expression to a measurable biological response. This was done by developing cell lines that were unique in their ability to mount an adaptive response. Cell lines were classified as non-adaptive or adaptive cells. The cell lines that were able to initiate an adaptive response for radiation-induced chromosome aberrations had a different gene expression profile than cells with the genetic make-up, which made them non-adaptive for the same endpoint (Coleman et al. 2005). All of the cells were exposed to 50 mGy and their genome evaluated for changes in gene expression. Of a total of 12,000 genes evaluated, the number of genes that had their gene expression significantly either up or down regulated in non-adaptive cells was 57. The number of unique changes in gene expression in adaptive cells was 45, and genes that changed gene expression in both cell lines after an exposure to 50 mGy totaled 47. These changes in gene expression are illustrated in Figure 14.

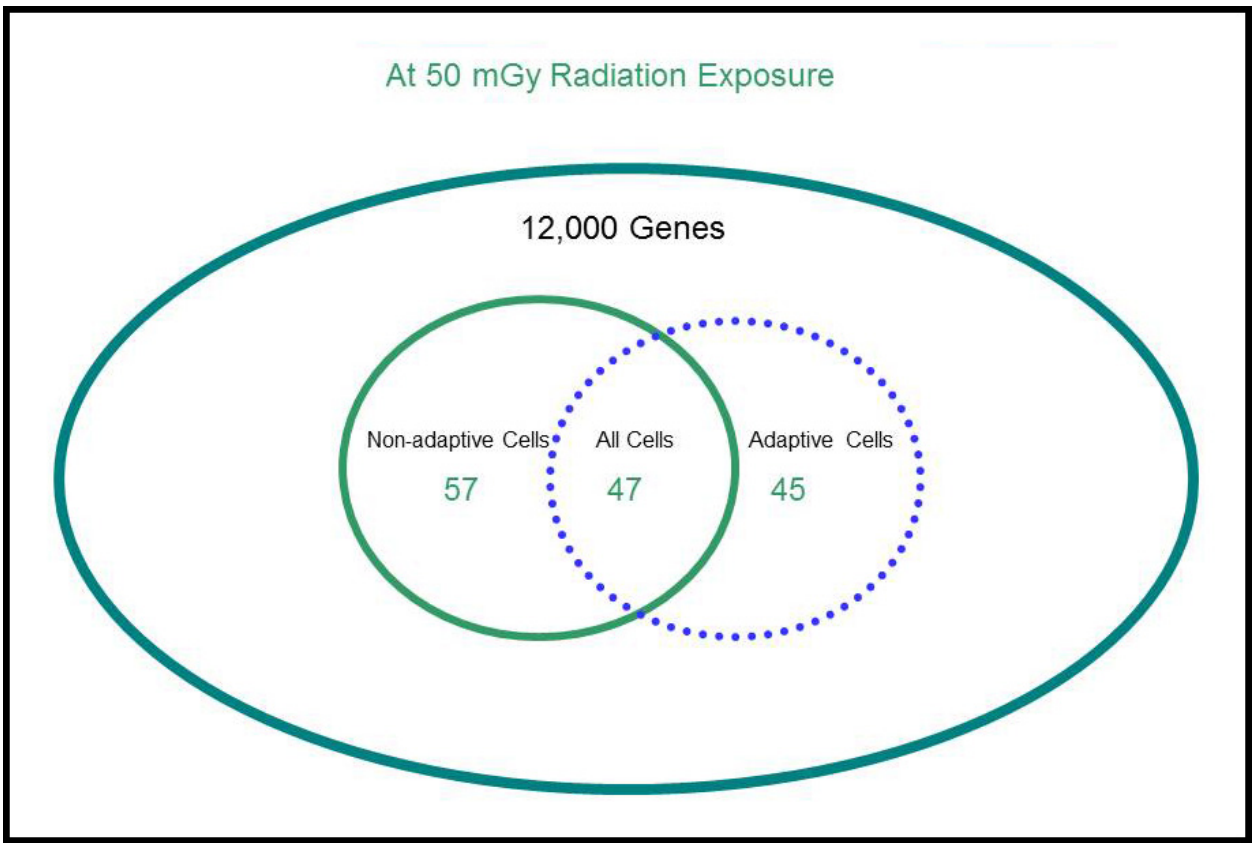


Figure 14. Radiation-induced changes in gene expression in adaptive and non-adaptive cells. Cells were given an adaptive dose of 50 mGy, and 12,000 genes were evaluated. A difference in gene expression was shown between adaptive and non-adaptive (Coleman et al. 2005).

The function of the unique genes in each of these cell lines were evaluated and placed into four categories: 1) genes that were up-regulated in all the cells, 2) genes that were down-regulated in all the cells, 3) genes that were up-regulated in adaptive cells and down-regulated in non-adaptive cells, and 4) genes that were down-regulated in adaptive cells and up-regulated in non-adaptive cells. It was determined that the third group included the genes involved in DNA repair and cellular responses to stress. The group four genes were associated with the induction of apoptosis and the regulation of cell cycles.

It has been well established in human skin that gene expression changes as a function of both dose and time after exposure (Goldberg et al. 2004). Several critical genes were evaluated in human skin biopsy as a function of distance from the treatment area for prostrate cancer as a function of both dose and time after exposure (Figure 15a and b).

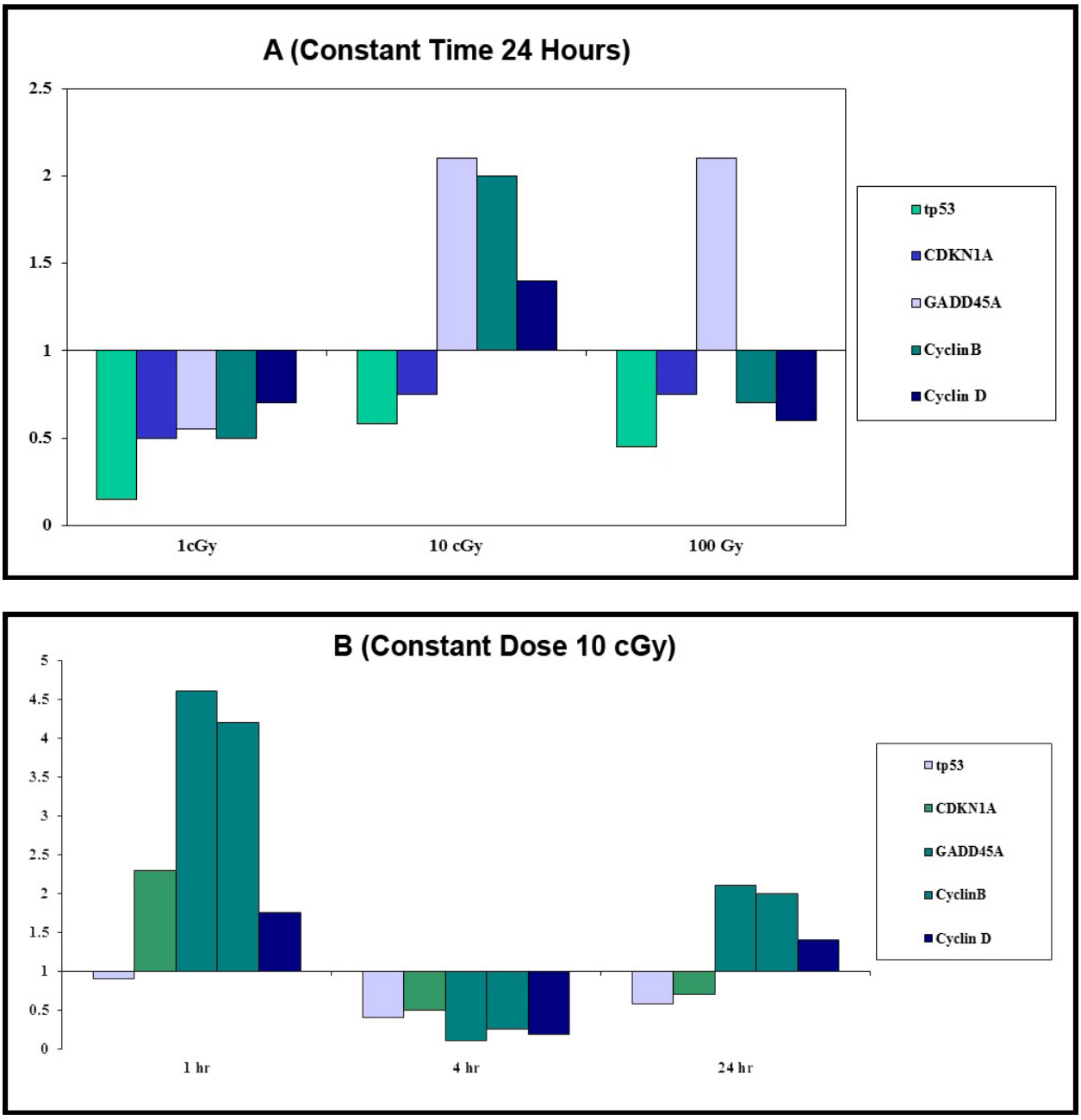


Figure 15. Gene expression changes as a function of both A) radiation dose and B) time after exposure (Goldberg et al. 2004).

These studies demonstrate that critical genes are both up and down regulated as a function of time after exposure and dose. These sets of genes were all down regulated at low dose 1cGy when sampled at 24 hours. However, the same set of genes were either not changed or up regulated when sampled at 1 hour after exposure to a 10-cGy radiation dose.

Such studies help identify the potential mechanisms of action associated with changes in adaptive responses. Other human studies have indicated that there are transient genome-wide changes in transcriptional responses following exposure to ionizing radiation (Berglund et al. 2008). Many of the changes in gene response are directly related to stress responses (Sheikh & Fornace 1999) . Thus, even following low doses of ionizing radiation the cells recognize exposure to ionizing radiation as a form of stress. Such stress responses have also been implicated in many processes related to aging. Radiation exposure of the brain demonstrated that many of the changes in gene expression could be related to changes in cognitive functions, Alzheimer's disease and other changes related to advanced aging (Lowe et al. 2009). However, there has been little evidence to document that radiation causes changes in the aging processes. This may be an area of fruitful future research.

It is important to remember the fact that gene expression and protein production and the lifetime of the proteins may not be linearly related. Relating gene expression to protein expression following radiation is an important research area (Coleman et al. 2000). The next important step is to relate changes in proteins to the development of cancer. This was done using a proteomics approach to study leukemia induction in mice (Rithidech et al. 2007a; Rithidech et al. 2007b). Various *in vitro* systems have been used to study the role of low doses in altering the spontaneous frequency of micronuclei, cell killing, and apoptosis (Sahijdak et al. 1994; Kagawa et al. 2001). It has been postulated that changes in apoptosis may be responsible for many of the adaptive responses seen following low doses of radiation and could differentially remove transformed cells from a cell population, reducing the background frequency of cell transformation and potentially reducing the risk for cancer in the low dose region below the spontaneous level (Bauer 2007a).

Although the field of radiation-induced apoptosis has expanded very rapidly at the time of the DOE Program, it will not be reviewed in detail here. The research conducted on radiation-induced apoptosis under the Program and its implications will be discussed. Apoptosis has been postulated to play an important role in adaptive responses for both the formation of cancer (Bauer 2007a) and mutation induction (Sykes et al. 2006a) . Building such relationships between the different cellular endpoints and the induction of the adaptive response provides critical information that is needed to link molecular changes, cellular changes, changes induced in whole animals to the cancer induction and risk in humans. The mechanisms for the induction of apoptosis show that this radiation-induced process plays an important role in cancer risks at low doses and will be further evaluated in Chapter 6.

Research has demonstrated that low doses of ionizing radiation can also have effects in whole animal *in vivo* studies. Individual chemicals changes induced by low doses of radiation show protective effects. This was demonstrated as radiation induced increases in apigenin reduced the frequency of chromosome damage in human lymphocytes (Rithidech et al. 2005). These studies demonstrated that low doses of radiation can depress the frequency of birth defects during embryogenesis (Wang et al. 2000). Low doses can also decrease the frequency and increase the time of onset of cancer in mice (Mitchel 2006). It has also been demonstrated that low dose-rates from low-LET radiation delivered to mice can result in a decrease in the frequency of cancer (Sakai et al. 2003). Low doses of low-LET radiation have been suggested to decrease the frequency of plutonium- or smoking-induced lung cancer by low doses of low LET radiation

(Sanders & Scott 2008). Exposure of dogs to internally deposited radioactive material that emit low-LET radiation resulted in no change in the frequency of lung cancer at total cumulative lifetime doses of less than 20 Gy (Brooks et al. 2009).

Radiation-induced adaptive responses are thus well established in molecular, cellular, and whole animal studies. However, to have an impact on standard setting it is important to demonstrate that such changes may exist in human populations exposed to low doses of radiation. The epidemiological studies of human populations exposed to low doses and dose-rates have been carefully reviewed (NRC 2006). The ability to determine the shape of the dose-response in the low dose region is very difficult using epidemiological methods (Shore 2009). Many studies of cancer incidence in populations exposed to elevated low dose-rates from natural background show no response, less-than-predicted responses, or small positive responses (NRC 2006) Other studies of nuclear workers exposed to low doses of radiation showed a small but insignificant increase above the background level. Thus, human studies of populations exposed to low dose or dose-rates from radiation suggest that there is an increase in risk from low dose exposures but fail to demonstrate either a significant positive or negative dose-response relationship.

However, "the risk from radiation exposure is highly quantifiable in terms of modifying factors such as age and sex, exposure to other carcinogens such as tobacco smoke and the measurable effects of other factors, usually unknown that influence variations in baseline cancer rates by populations" (Land 2009). The difficulty is in part related to the variables in the baseline to cancer lifetime radiation induced risk. The risk of cancer diagnosis in humans is about 45%, and lifetime risk of cancer mortality is about 25% (NRC 2006). Even in human populations that received a substantial radiation dose delivered at a low dose rate, controversy remains. Papers have been published stating that ionizing radiation delivered at a low dose rate can reduce the cancer frequency in humans (Chen et al. 2004a).

There have also been studies conducted that have mixed findings on the usefulness of the adaptive responses or low dose-rate exposure as a way to protect normal tissue during radiation therapy for cancer (Chen et al. 2004a; Redpath 2007). However, because most of these clinical studies were not conducted as part of the DOE Program they are not reviewed here.

The major points associated with radiation-induced adaptive response are summarized below and demonstrate that the adaptive response is a real and important biological phenomena that must be considered when evaluating radiation standards. If nothing else, the data demonstrate that the LNT hypothesis currently recommended for setting standards is conservative in the low dose region and that there is extensive well-documented scientific data on the responses in this region that support this statement.

Major Points: Adaptive response

There is a long and well-documented history of hormesis research that demonstrates many chemical and physical agents that produce damage at high doses, but illicit protective responses at low doses. Much research supports protective adaptive responses for low doses of radiation.

There are two major types of adaptive responses: 1) when a small tickle dose of radiation is given prior to a large radiation dose the response is less than if the large dose is given alone, and 2) low doses of ionizing radiation produce a reduction in the background frequency of many biological responses.

- The cellular and molecular responses following exposure to low doses of radiation are different from those induced by high doses, suggesting different mechanisms of action for high and low doses.
- The radiation-induced adaptive response is a very general biological phenomenon and has been carefully documented for many important biological endpoints including the induction of DNA damage, mutations, micronuclei, chromosome aberrations, cell killing, apoptosis, genomic instability, and cell transformation.
- The adaptive response has been demonstrated both *in vitro* and *in vivo*.
- The adaptive response suggests that there is a need for a change in the current paradigms associated with the LNT biophysical models used to estimate risk.
- The extensive data generated from research on the adaptive response suggests that following exposure to low doses of ionizing radiation, the LNT assumption is conservative.

III. Genomic Instability

A. Background

When cells are exposed to ionizing radiation, immediate changes that influence the genetic status of the cells are observed. Some of these include the induction of DNA breakage, changes in DNA bases, mutations, chromosome aberrations, and cell killing. These changes are related directly to the exposure conditions including dose, dose rate, and dose distribution. The frequency of these changes increases as a function of the radiation exposure and dose. The frequency of the radiation-induced changes is also dependent on the tissue where the changes are measured. After the initial response, the damage induced by the exposure decreases as a function of time after the exposure until the frequency of cells showing changes in the system returns to near normal levels of damage. Such phenomena have been investigated for many years in multiple systems and have been summarized nicely in thousands of publications. These genetic alterations are correlated with the induction of genetic disease and cancer.

It was noted that there are multiple genetic changes in many types of cancers that reflect the loss of genetic stability of the cells. This loss of genomic stability seems to be one of the hallmarks of the cancer process (Hanahan & Weinberg 2000) and is critical as the cells take on a cancer phenotype. Genomic instability is a marker of cancer and is widespread in many cancer types (Lengauer et al. 1998). However, it is not known if the genomic instability is actually induced by the agent (such as radiation) that “caused” the cancer or is simply a reflection of the cancer process where cells have escaped genetic control present in normal tissues.

Extensive research has been conducted that demonstrates the many ways that cells maintain their genetic stability. Research conducted in the DOE Program in this subject area has identified

many genes involved in control of the genomic stability of the cells. Specific genes have been identified that control against the induction of DNA cross linking (Liu et al. 1998). Other important genes that maintain genomic stability, including chromosomal stability, have also been characterized (Liu et al. 1998; Honma et al. 2000; Fujimori et al. 2001). For example, it has been shown that there are specific genes that control the processes involved in DNA repair that are essential in maintaining normal genomic stability (Thompson & West 2000). Studies were conducted using RNA interference to study DNA processing genomic stability, mutations, and cancer. All these processes seem to be linked to common pathways, with the repair of DNA damage being one of the critical pathway elements that leads to genetic damage (Bedford & Liber 2003). Genes involved in two of the major DNA repair pathways involved in genomic stability were identified. These include genes important in DNA excision repair (Amundson et al. 2002) as well as homologous recombinational DNA repair (Thompson & Schild 1999). These genes have all been shown to be essential for maintaining genomic stability. Other processes important in genomic stability involve the balance of the reactive oxygen species (ROS) status of the cells. Radiation-induced genes have been identified that control the ROS status of the tissues by increasing the free radical scavengers in cells (Limoli et al. 2001a).

Following radiation exposure, cells can make multiple, apparently normal cell divisions, then a fraction of the irradiated cells can lose control of their genome. In the field of radiation biology, this was unappreciated in the past. This "genomic instability" or loss of genetic control, results in multiple genetic changes in the cells. Genomic instability has been defined as the increased rate of acquisition of genetic alterations in the progeny of an irradiated cell (Morgan et al. 1996). These changes are similar to those observed a short time after exposure.

One of the earliest reports of genomic instability *in vitro* was related to the induction of DNA damage and its role on the induction of chromosome instability (Marder & Morgan 1993). In this system, CHO cells containing a human chromosome #4 (CM10114 cells) were exposed to radiation, clonally expanded and the progeny examined for the induction and change in type and frequency of chromosome aberrations. Radiation-induced late-occurring multiple genetic changes were first observed using an *in vivo/in vitro* method in the bone marrow of mice that were exposed to ^{239}Pu (Kadhim et al. 1992). The mice were exposed to ^{239}Pu and the bone marrow examined *in vitro* after several cell divisions in tissue culture for the presence of abnormal chromosomes. The frequency of chromatid aberrations was increased, showing that the aberrations were not being produced by direct exposure to the alpha particles from the internal emitter. Similar changes were identified in primary cultures of irradiated human bone marrow cells. In these studies, the genomic instability was measured as both an increase in apoptosis and as chromatid type aberrations (Kadhim et al. 1995).

After the initial observations of radiation-induced genomic instability both *in vivo* and *in vitro,* many systems were developed where the genomic instability could be quantified and carefully followed in individual cells. Genomic instability has been demonstrated in a range of different cell systems and cell types, suggesting that it is an important biological endpoint in the development of cancer. Genomic instability has been induced by radiation exposure in CHO cells that contained a copy of human chromosome number 4 (CM10115 cells) (Morgan et al. 1996; Morgan 2003c; Morgan 2003b), in mammary cells (Ponnaiya et al. 1997b), in mouse bone

marrow (Kadhim et al. 1992; Bowler et al. 2006), in human lymphocytes (Lou et al. 2007), and human bone marrow cells (Kadhim et al. 1995).

A wide range of different endpoints were used as a measure of the induction of genomic instability. One of the major endpoints identified was the change in the frequency and type of chromosome aberrations as a function of time after the radiation exposure in a way that was inconsistent with the development of clones of abnormal cells. Late-occurring chromosome aberrations thus became one of the standards for measuring genomic instability (Marder & Morgan 1993; Morgan et al. 1996; Ponnaiya et al. 1997b; Schwartz et al. 2001). This endpoint was then related to many other changes that were indicative of genomic instability in the cells (Romney et al. 2001). The induction of chromosome instability (Schwartz et al. 2001) was related to changes in DNA repair including defective recombinational repair (Takata et al. 2001) , DNA cross links (Donoho et al. 2003), and the induction of micronuclei and DNA damage measured by the comet assay(Lou et al. 2007). In addition to measuring chromosome instability, other endpoints of genetic instability were developed including delayed hyper-recombination (Huang et al. 2004), induction of lethal mutations (Mothersill & Seymour 1997) and changes in DNA copy number (Kimmel et al. 2008).

However, studies have been conducted suggesting that genomic instability cannot be induced in stable normal cells. This inability of radiation to cause genomic instability has been demonstrated in human cells (RKO cells) in cultures (Huang et al. 2007), as well as in other normal human and animal cell lines (Dugan & Bedford 2003). The failure to demonstrate genomic instability in normal cells suggests that it may be part of the process of the cancer development and not induced by the radiation insult. If this is the case, genomic instability would not be detected in A-bomb survivors that received the radiation exposure but had not developed cancer. There has been no genomic instability demonstrated in follow-up of the A-bomb survivors.

Studies have been published to try to resolve the differences in the induction of genomic instability seen in experimental systems and the failure to demonstrate it in normal cell populations and in human populations (Morgan & Sowa 2007). One study demonstrated that when normal human cells (RKO) were incubated with growth medium conditioned by cells that were genomically unstable, high doses of radiation (5Gy) could induce genomic instability. This demonstrated that in addition to the radiation, the growth medium was critical for the induction of genomic instability. Using this cell system, it was also determined that the frequency of radiation-induced genomic instability could be decreased by previous exposure to a low dose of radiation, a phenomenon indicative of an adaptive response (Huang et al. 2007). Thus, genomic instability and adaptive response appear to be closely related.

Studies were conducted to determine the cellular target for the induction of genomic instability. DNA damage (Morgan et al. 1996) and the cell nucleus (Kaplan & Morgan 1998) were implicated as the target for the induction of genomic instability. Studies on the induction of chromosome instability using different dose and dose-rates also defined the nucleus as the target for genomic instability (Limoli et al. 1999). More recent studies have extended these studies and demonstrated that genomic instability can be induced by exposing a single chromosome to

ionizing radiation (Mukaida et al. 2007). Thus, the target for the induction of genomic instability has been demonstrated to be the nucleus of the cell.

B. Physical Variables That Influence the Induction of Genomic Instability

One of the major concerns associated with the induction of genomic instability was the role of LET on this endpoint (Evans et al. 2003; Smith et al. 2003). This concern was related to the radiation environment in space where a range of different high Z particles, such as iron-56 and carbon, exist. These high Z particles contribute only a small fraction of the total energy deposited, but may be very important in evaluating the radiation risk related to space travel. It was of interest to NASA to determine the risk for exposure to these high Z particle in the space environment. Thus, a number of studies were jointly funded by the DOE Low Dose Radiation Research Program and NASA to extend the research from an interest in low-LET radiation to cover a range of different types of radiation.

Many of these studies of high Z particles were conducted at Brookhaven National Laboratory where high Z particles can be delivered over a range of well-defined doses and dose rates to both cells and whole animals. Research demonstrated that genomic instability was induced by many different types of radiation exposure including UV light (Durant et al. 2006), gamma rays and neutrons (Ponnaiya et al. 1997b), Carbon ions and X-rays (Hofman-Huther et al. 2006) and heavy ions like iron-56 (Limoli et al. 2000b; Evans et al. 2003). Studies were also conducted to determine the role of dose-rate on the induction of genomic instability. Over a limited dose-rate range there was little influence of dose rate on the frequency of genomic instability induced by high Z particles (Limoli et al. 1999).

Studies were conducted to define the dose-response relationships and the role of LET on the induction of genomic instability. The frequency of radiation-induced genomic instability was found to be high, about 3-4% increase in genomic instability per Sv, and similar following exposure to low-LET radiation, alpha particles or HZE particles (Limoli et al. 2000b; Limoli et al. 2000a; Evans et al. 2003; Kadhim et al. 2006). This very high frequency of genomic instability induced per unit of exposure suggests that the target for induction of genomic instability is much larger than a single gene and is not a simple mutation or combination of mutations.

The fact that a single dose of radiation can produce genomic instability suggests that genomic instability may be a potential mechanism to induce the multiple cellular changes required to change a normal cell into a cancer cell. Such information provides evidence that radiation-induced genomic instability may be a major pathway for a radiation-induced cancer. Over the dose range where significant increases in the frequency of genomic instability were observed there was a linear increase as a function of dose. Thus, radiation-induced genomic instability could provide a mechanism that would result in an LNT dose-response relationship for the induction of cancer.

However, because of the high signal-to-noise ratio, it was very difficult to determine the induction of genomic instablility in the low dose region. It was noted (i) that the control level of

genomic instability was higher than the level of genomic instability observed following low doses of radiation (<0.5 Gy, Figure 16) (Limoli et al. 2000b; Limoli et al. 2000a).

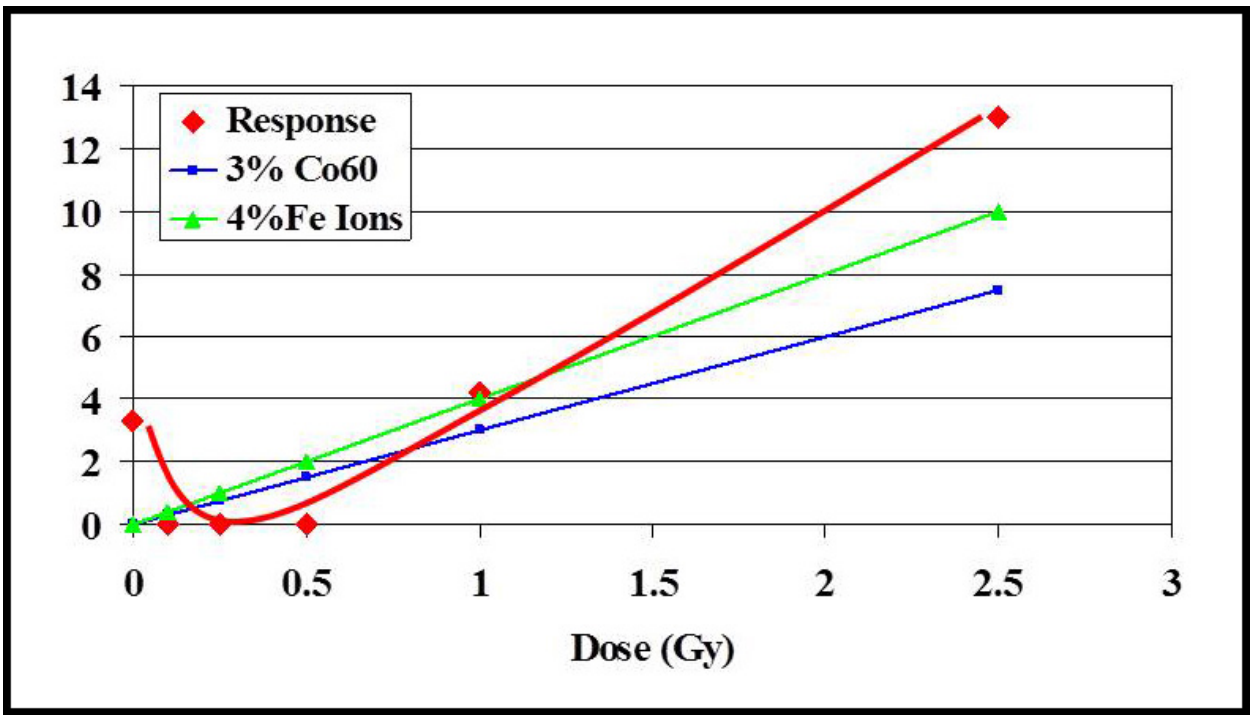


Figure 16. Percentage of cells with radiation-induced genomic instability following exposure to either low-LET cobalt-60 gamma rays or high-energy iron nuclei. A high frequency of genomic instability was induced by either treatment. Control level of genomic instability was higher than the level of genomic instability observed following low doses of radiation (<0.5 Gy) (Limoli et al. 2000b).

In these studies, linear dose-response models were used to estimate the frequency of induction of genomic instability and to extrapolate the results into the low dose region where significant responses were not seen. Thus, the data do not preclude the existence of non-linear dose-responses and the potential for an adaptive response in the low dose region for the induction of genomic instability.

As the data on the role of LET on the induction of genomic instability in bystander cells were reviewed and evaluated, it was found that radiation quality has a minimal effect on the frequency of radiation- induced genomic instability in bystander cells (Smith et al. 2003; Kadhim et al. 2006). However, all doses of high-LET radiation produced genomic instability in their cell systems while 3.0 Gy of low-LET radiation did not produce genomic instability.

C. Biological Processes That Influence Genomic Instability

1. ROS status of the cells

Early in the research on genomic instability it was recognized that oxidative stress played an important role in producing cells with chromosome instability. In genomically unstable cells, persistent increased oxidative stress was one of the physiological alterations noted (Limoli et al. 2003). In addition to oxidative stress, other cellular changes were observed such as apoptosis, cell cycle checkpoint modifications, and reproductive failure during cell division. Thus, changes in oxidative stress modified many cellular functions that ultimately resulted in chromosome instability (Limoli et al. 1998; Limoli et al. 2004).

It was determined that stress from non-DNA damaging agents could also result in genomic instability (Li et al. 2001). Because the mitochondria are the major organelles involved in production and maintenance of the proper level of reactive oxygen in the cell, studies were conducted to evaluate their function in cells that demonstrated genomic instability (Kim et al. 2000). These studies showed that mitochondrial dysfunction was a landmark of genomically unstable cells. This suggested that in addition to modifications in DNA, modification of the mitochondria also plays an important role in genomic instability. The modification of radiation-induced oxidative stress by free radical scavengers and cell proliferation was shown to prevent the induction of genomic instability (Limoli et al. 2000b; Limoli et al. 2001b). These observations and others associated with the mechanisms involved in induction and maintenance of genomic instability are discussed in chapter 6 and suggest that the oxidative status of the cells is one of the most important factors involved in maintaining the genomic integrity of cells. This suggests that there are important non-DNA mechanism involved in the induction and modification of genomic instability. Research suggests that reactive oxygen and oxidative stress in cells represent one of the major mechanisms involved in many of the radiation-induced changes associated with low dose exposures.

2. Apoptosis

Extensive research has been conducted in the past few years on radiation-induced apoptosis. This is an important mechanism involved in maintaining the genomic stability of cell populations by eliminating damaged cells. In fact, variation in the frequency of apoptosis was determined to be another marker of genomic instability (Nagar & Morgan 2005). Both delayed apoptosis and the induction of genomic instability were present following exposure to Carbon ions (Hofman-Huther et al. 2006) .

3. Death-inducing effects (DIE)

During the study of genomic instability in cell systems, it was discovered that soluble factors were secreted into the medium by genomically unstable cell lines that had a marked effect on the survival of normal cells (Nagar et al. 2003b) . These factors were called death-inducing effects (DIE). Clones of cells with radiation-induced genomic instability released unique substances into the media. Very small concentrations of the media from the genomically unstable cell lines would result in 100% lethality in the normal cells. The role of these factors on the induction of genomic instability and the potential impact of DIE factors on radiation-induced cancer have

been reviewed (Nagar & Morgan 2005). Because these factors did not seem to be produced by either normal or tumor cells research limited research or publications exist on the role of DIE on genomic instability or during the induction of cancer.

Genetic background, cancer and genomic instability

To link genomic instability to cancer, several important studies were conducted demonstrating that animals displaying an increase in genomic instability were also sensitive to cancer induction. From the early discovery of radiation-induced genomic instability, it was evident that the genetic background of the system being studied had a marked influence on the outcome of the studies. One of the earliest and best studies linking genetic background to the induction of genomic instability and cancer was conducted by exposing different strains of mice with different sensitivity to radiation-induced mammary cancer to radiation and measuring both the induction of genomic instability and mammary cancer in these mice. BALB/c mice with a high frequency of spontaneous of mammary cancer were used along with C57BL/6 mice in which mammary cancer was not seen in the controls. In these studies, the frequency of radiation-induced mammary cancer was very high in BALB/c mice and low or non-existent in the C57BL/6 mice. The frequency of radiation-induced genomic instability was also high in the BALB/c mice and low in the C57BL/6 mice (Ponnaiya et al. 1997a, b).

Figure 17 demonstrates that after a number of cell population doublings, the cells of the BALB/c mice demonstrated a marked increase in chromosome aberrations as an indication of the induction of genomic instability while the C57BL/6 mice had no increase in chromosome damage. These observed strain differences made it possible to link genomic instability to the genetic background of the animals and the induction of cancer.

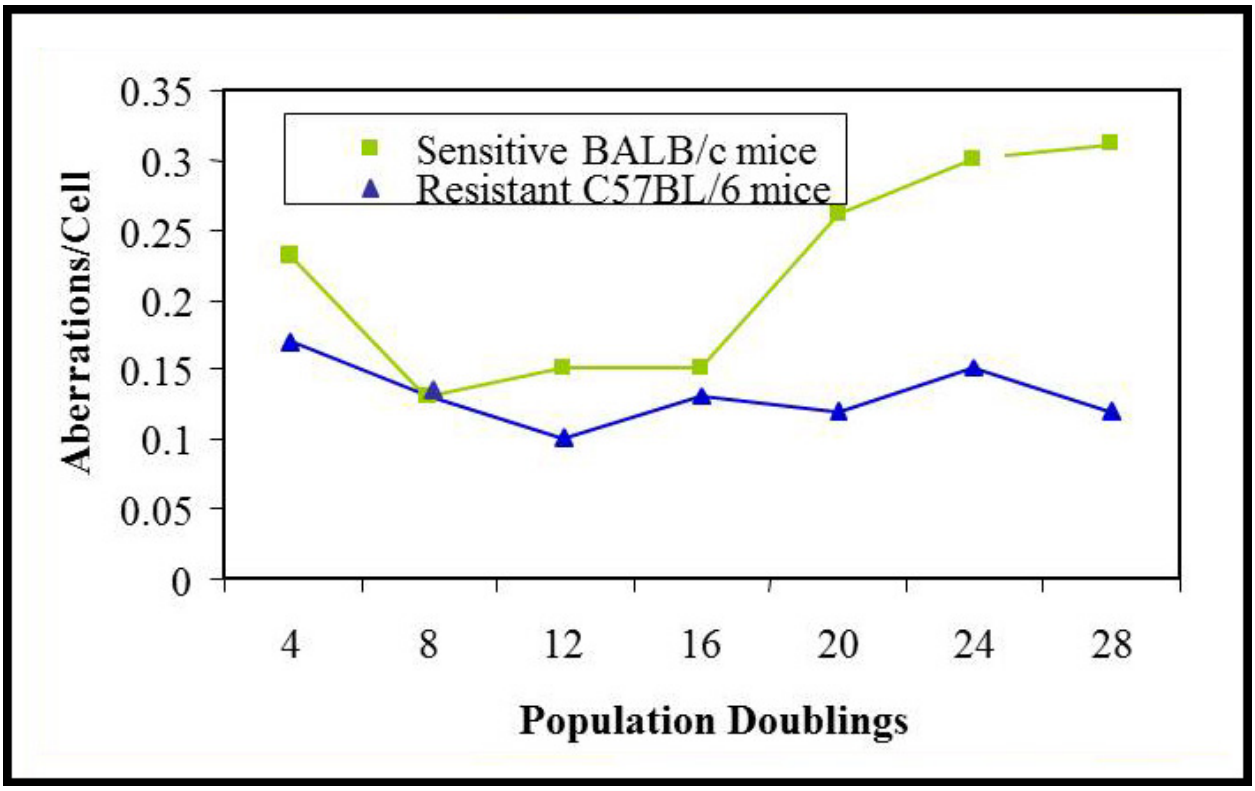


Figure 17. Demonstration of the lack of genomic instability in C57BL/6 mice and the induction of genomic instability in radiation-sensitive BALB/c mice. The genomic instability was seen after ~16 cell population doublings (Ponnaiya et al. 1997a).

Additional studies using the mammary epithelial cell system confirmed that p53 null mice could be induced to have chromosomal instability related to changes in hormonal status. This genotype seemed to be in part responsible for the difference in the biological responses observed between the sensitive (BALB/c) and resistant (C57BL/6) mouse strains to radiation-induced cancer and genomic instability (Pati et al. 2004). The importance of the TP53 gene in genomic instability was also demonstrated using other cell lines, including human lymphoblastoid cells (Schwartz et al. 2003). The genomic instability demonstrated in lymphoid cells seemed to play a role in the induction of mouse lymphoma in p53 heterozygous mice (Mao et al. 2005).

Other geneotypes associated with DNA damage repair were identified as important during the induction of genomic instability. Extensive studies were conducted and reviewed to evaluate the role of DNA repair deficient mutants in CHO cells (Somodi et al. 2005). It was determined that defective recombinational repair in 5 Rad 51 paralogs were involved in genomic instability (Takata et al. 2001). Genomic instability was also demonstrated in Gadd45, a deficient mouse strain (Hollander et al. 1999). Rad51C deficiency was shown to destabilize XRCC3, impair recombination, and increase radiosensitivity in cells in the S/G_2 stages of the cell cycle. Major changes in DNA crosslinking, chromosome instability, and mice lifespan were associated with alterations in Braca2 exon 27 gene (Honma et al. 2000). This research all points toward DNA damage and repair in the nucleus as the target for radiation-induced genomic instability. Finally,

cells and tissues from people with genetic diseases such as Xeroderma pigmentosum, which is influenced by p53 (Cleaver et al. 1999a), and retinoblastoma (Zheng et al. 2002) were found to display genomic instability. The importance of genetic background on the induction of genomic instability has been reviewed (Kadhim 2003).

Genomic instability has been related to other types of radiation-induced cancer in experimental animals. Exposure of mice to either radiation or benzene produced genomic instability and acute leukemia (Rithidech et al. 1999). Studies demonstrated that radiation could also produce genomic instability in the lymphocytes and lymphoma in p53 heterozygous mice (Mao et al. 2005; Bowler et al. 2006). These studies have established an association between cancer and genomic instability and have strengthened the link between genetic background and genomic instability. Such research also suggests that genomic instability is an important part of the radiation-related process as cells and tissue progress from normal to cancer.

Review papers have been written on radiation-induced genomic instability (Little 1999, 2003; Morgan 2003b, a) that provide additional information and will help complete this literature review.

A number of papers have used the data to link genomic instability to radiation-induced cancer in humans (Goldberg & Lehnert 2002; Huang et al. 2003). These papers make a convincing argument that radiation-induced genomic instability could represent an early stage of radiation-induced cancer and may be important in the cancer process for many forms of cancer displaying genomic instability. Finally, it will be of interest to determine if the induction of genomic instability can be modified by low doses of radiation and other environmental insults and what, if any, the clinical implications of this process are (Goldberg 2003).

5. Epigenetics and genomic instability

Recently the role of epigenetic factors, or those not associated with direct changes in the DNA or genetic material, has been investigated. Early research demonstrated that changes in diet of special strains of mice can change the coat color of the offspring. These observations have been expanded to other systems to help understand the risk from exposure to a number of different types of environmental insults such as diet and environmental pollutants (Jirtle & Skinner 2007). A mother's diet can modify the environment of a fetus during development, changing gene expression and modifying phenotype. It has been suggested that epigenetic influences during development may play an important role in the development of cancer and other genetic diseases in adults (Dolinoy et al. 2007b; Dolinoy et al. 2007a). Environmental exposures to a number of different insults can change the offspring in a way that suggest that epigenetic mechanisms could play an important role in human health and disease (Dolinoy & Jirtle 2008).

The role that radiation plays in induction of epigenetic changes is a relatively new field and requires additional research. However, two research areas suggest that in addition to causing direct alterations to the genetic material, radiation also plays an epigenetic role in causing phenotype modifications and cancer risk without direct interaction with the DNA or chromosomes.

First, it has been suggested that the ability of radiation to modify non-genetic targets such as the extracellular matrix and influence the outcome of diseases plays a role in the induction of breast cancer (Asch & Barcellos-Hoff 2001). It has been demonstrated that radiation exposure of the stroma, which is connective tissue and contains no genetic information, has been linked to an increase in the frequency of breast cancer (Barcellos-Hoff & Ravani 2000). Such studies suggest that a tissue is responding as a whole to the exposure to radiation and not as a set of individual cells.

Second, it has been suggested that radiation can induce genomic instability through epigenetic mechanisms that could be passed from one generation to the next. Studies were conducted using a mouse model system where chimeric male mice were exposed to radiation and their radiation history was traced to the F(2) generation. Changes were shown in gene expression and enzyme activity in the many kinases in organs of offspring where the parents had been exposed to radiation (Baulch et al. 2002; Vance et al. 2002). These changes are indicative of the induction of genomic instability in the offspring and suggest the potential for increased cancer risk in the radiated mice.

Other studies with the same system were initiated that indicated radiation-induced cellular reprogramming resulting in changes in gene and protein expression. These changes were followed in the offspring through the F(3) generation (Vance et al. 2002). These studies suggested that genomic instability had been induced in the offspring of the irradiated mice. The frequency of the changes in the offspring were high so that they could not be explained based on known genetic transmission and could only be explained by epigenetic mechanisms.

Epigenetic effects of radiation have been suggested in human and mouse populations exposed to either external radiation or from internally deposited radioactive materials. The role of radiation during the induction of epigenetic changes was reviewed early in the DOE Program (Nagar et al. 2003a) with little evidence of a marked effect on genetic or carcinogenic risks from these mechanisms (Nagar et al. 2003b). However, transgenerational epigenetic changes have been detected using changes in gene sequences in tandem repeated DNA loci (TRDLs). The high frequency of these changes suggests that they are induced by a mechanism different from that seen for direct radiation effects. These are measured in mini-satellites in human and expanded simple tandem repeats in mice.

A review of the literature involving the TRDLs noted important differences between the structure of mouse and human TRDLs and suggested that transgenerational effects associated with TRDLs are present in mice but may not be present in humans (Bouffler et al. 2006). Again, there is little evidence that these radiation-induced changes in TRDLs will play an important role in the risk for cancer or mutations in humans. The role of radiation-induced epigenetic effects is an important area and requires additional research because it could be one of the few areas where the risk from radiation-induced damage could have been underestimated.

6. Genes and proteins involved in genomic instability

In studies of adaptive response, extensive research was conducted on the genes and proteins involved in the induction of these protective effects. These studies were important for understanding the mechanisms involved. However, there has been only limited research on the

changes in gene expression and protein expression in cells demonstrating genomic instability. Research in this area could provide critical information on the role of genomic instability, especially the role of epigenetic effects, on cancer risk. Limited studies were undertaken to determine if changes in gene expression would provide clues for understanding the process of genomic instability (Snyder & Morgan 2004a, b). As the research continued, there was a lack of consensus of gene expression changes associated with radiation-induced chromosomal instability, and it became obvious that many processes may be involved during the induction of genomic instability.

With the development of new systems for rapidly detecting genomic instability using a stable transfected plasmid-based green fluorescent protein assay that detects homologous recombination and delayed mutation/deletion events (Huang et al. 2004), it became possible to measure radiation-induced genomic instability after low doses of radiation. Research on the mechanisms involved in the induction of genomic instability will be covered in more detail in Chapter 6.

Major Points: Genomic Instability

- Many cancers display genomic instability.
- Radiation produces genomic instability in a number of *in vitro* and *in vivo* experimental systems.
- Radiation-induced genomic instability is a frequent event. This suggests that it is not a process involving a single gene or small numbers of genes but requires a larger target for its induction.
- The nucleus of the cells seems to be the target for the induction of genomic instability.
- The ROS status and mitochondrial metabolism play critical roles in the loss of genomic stability.
- Some genomically unstable cell lines secrete a substance (Death Inducing Effect) DIE that is lethal to normal cells. To date this factor or substance has not been characterized.
- The genes and proteins involved in the induction of genomic instability are not well defined at this time.
- The genetic backgrounds of cells, animals, or humans are important in radiation-induced genomic instability.
- Animals that are sensitive to radiation-induced cancer are also sensitive to the induction of genomic instability.
- Evidence exists that small doses of radiation can produce an adaptive response for genomic instability and reduce genomic instability induced by large radiation doses.
- The presence of adaptive responses indicates a non-linear dose-response relationship for the induction of genomic instability. Nevertheless, genomic instability is a process that is triggered by a number of environmental factors and could potentially support the linear-no-threshold theory.

- Research is needed in the low dose region to further define the mechanisms of action and the shape of the dose-response relationship for induction of this important process.

IV. The Relationships of New Paradigms

The interrelationships between each of these new phenomena, bystander effects, adaptive response and genomic instability has been carefully documented (Sawant et al. 2001b; Lorimore et al. 2003; Zhou et al. 2003; Mitchell et al. 2004c; Lavin et al. 2005; Zhu et al. 2005; Hall 2006; Prise et al. 2006b), and is illustrated in Figure 18.

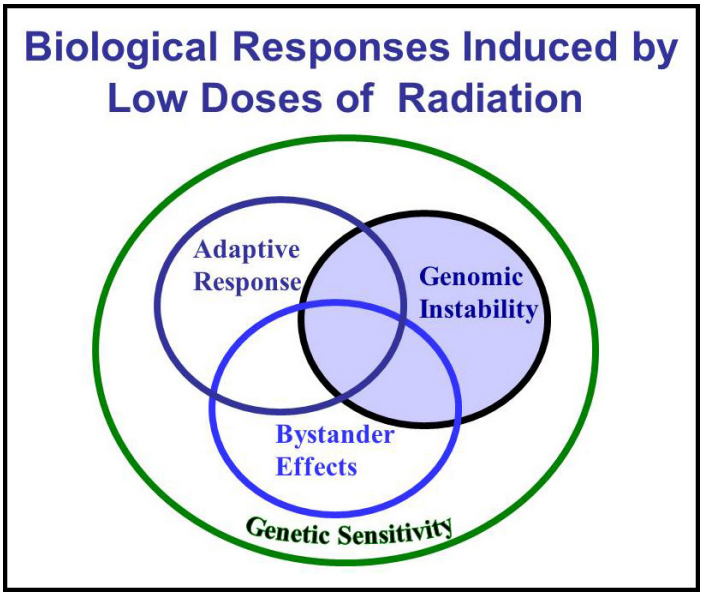


Figure 18. The overlap of biological responses induced by low doses of radiation. All these responses are dependent on the genetic background of the test system.

Each phenomenon is influenced by the genetic background and the genetic sensitivity of the system used to make the measurements. This is illustrated by the large circle over the whole figure. The close relationships that have been demonstrated between these seem to reflect manifestations of similar basic biological changes, and as studies have been expanded, adaptive responses, genomic instability, and bystander effects have been tightly linked.

A. Bystander Effect and Genomic Instability

Early in the Program it was recognized that there were many biological changes present in bystander effects and genomic instability, many of these changes could be related to the induction of cancer, and the critical molecules and pathways were influenced in measurements of each of them (Barcellos-Hoff & Brooks 2001). The research on the relationships that exist between bystander effects and the induction of genomic instability has been recently reviewed (Maxwell et al. 2008). Much of this literature has focused on how this interaction could potentially increase the radiation risk by causing genomic instability in cells with no energy deposited in them (bystander cells) (Morgan 2002; Morgan et al. 2002; Hall & Hei 2003;

Lorimore et al. 2003; Huang et al. 2007). This research suggested that the target for the induction of genomic instability was much larger than the number of cells traversed by radiation.

The response of bystander cells to produce genomic instability has been related to many physiological changes such as the induction of inflammatory-type responses induced by radiation (Lorimore & Wright 2003). It was suggested that secreted factors were important and research conducted to try to identify the important factors (Sowa Resat & Morgan 2004). The interactions between genomic instability and bystander cells have been the subject of a number of review articles (Lorimore et al. 2003; Maxwell et al. 2008).

B. Bystander Effects and Adaptive Responses

Early in the DOE Program much of the research was conducted *in vitro* using a wide range of different cell types. As bystander effects; the importance of cell-cell, cell/matrix, and cell tissue interactions; and total tissue responses became better understood (Bissell & Barcelloshoff 1987), the calls for proposals and the research direction in the Program shifted to the study of organized cellular systems and tissues. Many biological endpoints were measured that demonstrated the bystander effects and suggested that this response represents cell-cell and cell/matrix communication capable of altering biological outcomes (Barcellos-Hoff 2005a; Andarawewa et al. 2007b).

This research demonstrated that many of the responses observed in a mono-layer tissue culture were not observed in complex tissue. It also suggested that in complex tissues and whole animals other responses seemed to modify the observed biological responses on the single-cell level. It was demonstrated that adaptive responses are observed in cells that do not have energy directly deposited in them and that this could reduce the biological response (Schettino et al. 2005).

The control and modification of these single-cell processes by the environment had a major impact on the responses in the low dose region and helped to demonstrate that interactions between different cell types and modification and processing of the initial damage controlled the induction of radiation-induced disease. These processes need to be carefully considered as models of radiation risk are developed.

As the result of this research, it has been suggested that adaptive processes might be in play during radiation therapy for cancer (Sgouros et al. 2007). With further research, clinical applications of adaptive responses and bystander effects may provide mechanistic understanding of the responses to low doses of radiation. The use of low dose protocols and the induction of adaptive responses and bystander effects may be useful to modify cancer risk and adverse acute effects produced during cancer therapy.

C. Genomic Instability and Adaptive Response

It has been suggested that genomic instability may increase the radiation-related cancer risk above the level predicted by the LNT, while adaptive responses may decrease the calculated risk in the low dose region. Research has been conducted to determine the relationships that exist between these two observed phenomena. In cells that display genomic instability interleukin 8 was shown to produce a pro-mitogenic and pro-survival effect to modify radiation-induced

genomically unstable cell lines (Laiakis et al. 2008). (Dziegielewski et al. 2008) also demonstrated that chemicals that serve as radioprotectants such as WR-1065 act through their active metabolite, amifostine, to mitigate radiation-induced genomic instability.

These findings demonstrate potential protection against the induction of genomic instability and may also result in decreased incidence of radiation-related cancer. Research was conducted by (Huang et al. 2007) to determine if a small radiation dose prior to a large challenge dose used to demonstrate adaptive responses could decrease the frequency of radiation-induced genomic instability. They determined that previous low doses of radiation resulted in an adaptive response that decrease the response to subsequent high doses of radiation of the induction of genomic instability. Such research provides strong associations between these two biological phenomena and demonstrates the potential for adaptive responses to modify genomic instability as well as the risk for cancer.

Figure 18 suggests that all three of these observations are closely linked. Published studies suggest reasons for these tight links and associations, one important one being related to the fact that there is biological variability in all biological systems and that what was being measured was only an indication of this variability (Schwartz 2007). Many changes in gene expression, metabolic pathways, biological processes, and individual chemicals altered by low doses of radiation were suggested to be common in all these observations (Morgan 2003b, a). This suggests common mechanisms are involved in each process.

As research has progressed, a need has arisen for a much more mechanistic approach (Kadhim et al. 2004). Many of these common mechanisms will be reviewed in Chapter 6 by discussing the different mechanisms involved and how they impact the biological responses.

As the data have matured, it is obvious that bystander effects, genomic instability, and adaptive responses are seen in many biological systems and represent the body's attempts to deal with low doses of ionizing radiation. These discoveries have impacted current knowledge in the field of radiation biology and must be considered when discussing radiation effects, radiation protection, and predicting radiation risk in the low dose region.

This chapter has described these observations in many different systems to demonstrate that they are general biological phenomena and represent well-described biological responses to low doses of radiation. Chapter 6 will examine the biological responses and chemicals involved in low dose radiation responses in greater detail and review the recent data that suggest the mechanisms of actions and the biological basis of these observations.

V. Biomarkers of Radiation Exposure and Dose

Physical dosimetry is essential to accurately estimate radiation exposure and dose both from external radiation exposure and internally deposited radioactive material. However, there are cases where this is not possible. When the dose is from internally deposited radioactive material, like radon gas, and no direct measurement of dose is possible, the measurement of biological changes can often be used to generate data for models to estimate radiation dose (NRC 2006). In addition, there have been a number of radiation accidents where no physical dosimetry was

available. In such cases it was necessary to use biological changes in the exposed individuals to estimate radiation exposure or dose. Using biological changes to estimate dose after exposure is the field of biodosimetry. Biomarkers of radiation exposure, dose, and sensitivity have been successfully applied to a wide number of situations including radiation accidents.

When the dose is high, symptomatic markers, such as onset of nausea and vomiting, provide a first guess as to the dose received. At lower doses, other changes must be measured, such as the kinetics of lymphocyte depletion, where it is possible to relate the decline in lymphocyte numbers to the radiation dose and dose-rate (Goans et al. 2001; Dainiak et al. 2007). At still-lower doses, the gold standard for biodosimetry is the induction of chromosome damage. The use of chromosome damage as a biomarker of radiation dose for internally deposited radioactive materials (Brooks et al. 1997) and external acute radiation exposure (Bender et al. 1988) was a subject of extensive research before the Program was initiated.

The Program has not focused on biodosimetry or the measurements of biomarkers of radiation-induced changes, because their major use has been to estimate dose following radiation accidents, terrorist activities, or nuclear war. All of these events focus on saving lives following very high radiation doses.

Even though the focus of the Program was not on biomarkers, its research has resulted in major scientific advances involving development of new biomarkers that could be used to provide rapid estimates of radiation dose. This research provided a foundation for additional studies using cell and molecular changes as radiation exposure and dose biomarkers.

It was recognized early in the Program that biomarkers could be used to help define genetic background related to the induction of disease, as well as radiation dose (Albertini 1999). It became apparent that biomarkers that change as a function of radiation can measure different processes, so that in some cases the biomarker is a measure of radiation dose, in other cases a marker provides an indication of radiation sensitivity, and finally, the marker may indicate presence of a disease (Brooks 1999).

Chromosome aberrations are a very reliable biomarker of exposure and dose. However, they vary markedly between tissues (Bao et al. 1997), and the frequency of chromosome aberrations in a tissue is not necessarily related to cancer frequency in these tissues. In the past, chromosome aberrations and other biomarkers of radiation exposure have also provided a quick *in vitro* and *in vivo* method to determine the relative biological effectiveness (RBE) of many radiation types (Brooks et al. 1997; Groesser et al. 2007). These values were then used in radiation protection standards to estimate cancer risk. However, research from the Program has shown that although chromosome aberration frequency reflects dose, in many cases it may not be a direct reflection of cancer risk (Brooks 2003).

As biomarker research has developed, its importance has been recognized by the scientific community. Other agencies have initiated extensive funding on biomarkers for rapid evaluation of exposure and dose in mass exposure events. Centers have been developed for biomarker research and development of techniques providing rapid and reliable measures of exposures of large populations to high radiation doses. For example, the “Center for High Throughput

Minimally-Invasive Radiation Biodosimetry" was formed at Columbia University College of Physicians and Surgeons to apply modern radiation biological techniques to detect biological changes as a measure of radiation dose. In addition, special meetings have been held on biomarkers of radiation exposure that summarize the state of the art for biomarkers. These meeting were sponsored by organizations such as the National Council on Radiation Protection and Measurements (NCRP) (Brooks 2001), the military (Blakely et al. 2002) and NASA (Straume et al. 2008). Only the research conducted under the DOE Program that has had an impact on the field of biomarkers and biodosimetry will be briefly reviewed here.

A. DNA Damage

It has been well established that radiation can produce many changes in DNA. However, in the past, this damage was detectable only after high radiation doses. Development of techniques that detect damage and repair of DNA at specific loci and the production of γ H2AX foci made it possible to detect DNA damage in the low dose region (Burma et al. 2001). These techniques have been improved (Nakamura et al. 2006) and automated using such techniques as flow cytometry (Kataoka et al. 2006), making it possible to use them as markers of radiation exposure. Such techniques are very useful for detecting acute radiation exposure; however, there is some question as to their usefulness following exposure to low dose rates of ionizing radiation. In a study where the radiation was delivered at a low dose rate, there was little change in the frequency of γH2AX, even following larger (up to 5 Gy) radiation doses (Ishizaki et al. 2004).

Development and use of clustered lesions in the DNA, described in Chapter 4, were very useful in evaluating DNA damage over a wide range of radiation doses. These lesions have been suggested to be unique for radiation exposure, can be detected at relatively low exposure levels, and could be used as a biodosimeter to estimate radiation doses (Sutherland et al. 2001c).

B. Changes in Gene Expression

A major spinout from the Program has been the use of radiation-induced molecular changes to develop biomarkers of radiation exposure and dose. It was determined early in the Program that changes in mRNA expression can be measured as a function of radiation dose and may be useful in identifying individuals or populations exposed to ionizing radiation (Amundson et al. 2000). Continued research on radiation-induced changes in gene expression, especially studies following low dose and dose rate exposures, validated these changes as markers of radiation dose and exposure (Amundson et al. 2001b; Amundson & Fornace 2001). This resulted in a strong database relating dose to responses that was used to develop molecular biomarkers of radiation exposure and dose (Amundson et al. 2001a; Coleman & Wyrobek 2006).
Such research was validated by measuring changes in gene expression using human radiotherapy populations exposed to known doses of radiation during their medical treatment (Amundson et al. 2004). It was found that gene expression changes as a function of the genes measured, as well as the radiation dose, dose-rate, and time after exposure. This makes it difficult to use the change in a single gene as a marker of radiation exposure. Thus, clusters of genes and selected gene changes and times after exposure were selected to develop biomarkers for rapid detection of radiation exposure in large populations. There were, however, suggestions that permanent signatures in the genome and changes induced in the expression profiles that could potentially be used as biomarkers (Hande et al. 2003).

A large project was focused at determining the usefulness of gene expression in predicting survival of cells in culture. In this study, 60 cell lines that are used at the NIH have been evaluated for both survival and changes in gene expression and it was determined that different subgroups of genes may provide an indication of the radiation sensitivity of cells and tissues (Amundson 2008).

As this type of research was expanded, it has been funded by other government agencies that were interested in rapid biomarkers of radiation exposure in the event of nuclear war or terrorist attack. Research in the Program continues to be important in the development of molecular biomarkers of radiation exposure and dose but is not a central focus of the Program.

C. Changes in Proteins and Metabolites

Scientists recognized early on that changes in gene expression measured by changes in mRNA levels often may not reflect changes in physiological function. Proteins produced by the RNA are the important variable to determine changes in cell function. Therefore, it was important to measure protein changes induced by ionizing radiation and study the potential use of protein changes as biomarkers of radiation exposure. Some of the early work in this area was the result of basic research on the proteins involved in apoptosis or cell death called clusterin (Leskov et al. 2003). It was determined that changes in clusterin could be used as an indication of past radiation exposure (Klokov et al. 2003). With the development of protein microarrays (Coleman et al. 2003a), it became possible to rapidly measure radiation-induced changes in several proteins. This was an important factor in the development of protein changes as biomarkers of radiation exposure (Marchetti et al. 2006). Currently, many of these changes are detected only after doses of >2.0 Gy. In addition to changes in total protein, it was noted that radiation exposure changed the phosphorylation pattern and degree in human melanoma cells (Warters et al. 2007). Proteomics research is another important future area of research.

Finally, it has been suggested that radiation can induce changes in normal metabolism, which results in a change in metabolites in the urine or other body fluids (Tyburski et al. 2008). This has led to the development of the field of metabolomics. Again, because most of these changes are detected after high doses of radiation, this has not been a major emphasis in the Program.

D. Chromosome Aberrations as Markers of Radiation Exposure

Because the DOE Program is focused on low doses, a primary biomarker of radiation exposure studied by the Program has been the induction of chromosome aberrations. In the past, chromosome aberrations were evaluated as structural changes in metaphase cells, with a focus on the induction of dicentric chromosomes where two chromosomes are joined and have two centromeres and a pair of fragments (Bender et al. 1988). This endpoint was relatively easy to score by trained cytogeneticists and provided a very good estimate of radiation dose regardless of whether the exposure was *in vivo* or *in vitro*. This made it possible to construct very good dose-response relationships for a wide variety of different exposure types, tissue types and species. However, the Program's focus has been on understanding the mechanisms involved in the induction of chromosome aberrations.

The major advance in this area has been the development of a technique called fluorescence in situ hybridization (FISH) that make it possible to paint each chromosome a different color. Without FISH it was not possible to detect complex chromosome rearrangements, and many of the radiation-induced aberrations went undetected. Using such techniques, it became possible to detect complex chromosome aberrations where multiple chromosomes are involved *in vivo* (Rithidech et al. 2007a) and *in vitro* (Cornforth 2001) and to construct dose-response relationships.

Researchers determined that the frequency of complex chromosome aberrations changes as a function of dose following exposure to gamma rays (Loucas & Cornforth 2001). Other exposure variables, such as dose-rate, radiation type, and LET of the radiation exposure, also impacted the frequency and type of complex chromosome aberrations produced. The frequency was higher following exposure to alpha particles than was seen for exposure to gamma rays and was not as dependent on dose rate (Cornforth et al. 2002a).

However, it was determined that even at low doses and dose rates a single track of low-LET radiation can induce complex exchanges with up to four breaks involved. This observation may suggest a different mechanism for the induction of multiple chromosome aberrations in a single cell. Such a mechanism could involve the induction of genomic instability and the loss of genetic control. It may also help explain the presence of rogue cells, cells with multiple aberrations seen in a number of different studies of internally deposited radioactive material. These observations may represent the earliest signs of the induction of genomic instability.

Additional chromosome painting techniques have been developed that make it possible to paint and produce multi-color banding of the chromosomes so that many local regions in each chromosome can be marked (Brenner 2004). Using these techniques, small losses and insertions as well as inter-and intra-chromosome exchanges, can be detected (Brenner 2004). Such chromosome banding techniques, make it possible to measure small and subtle chromosome alterations that cannot be detected in individual chromosomes with other techniques.

The interchromosome inversions in Figure 19 show where a piece of the chromosome has been inverted and reinserted into the same chromosome. Figure 19c demonstrates that an individual small piece of a chromosome can be lost or added to individual chromosome. None of these aberrations would have been detected using either normal staining or chromosome painting.

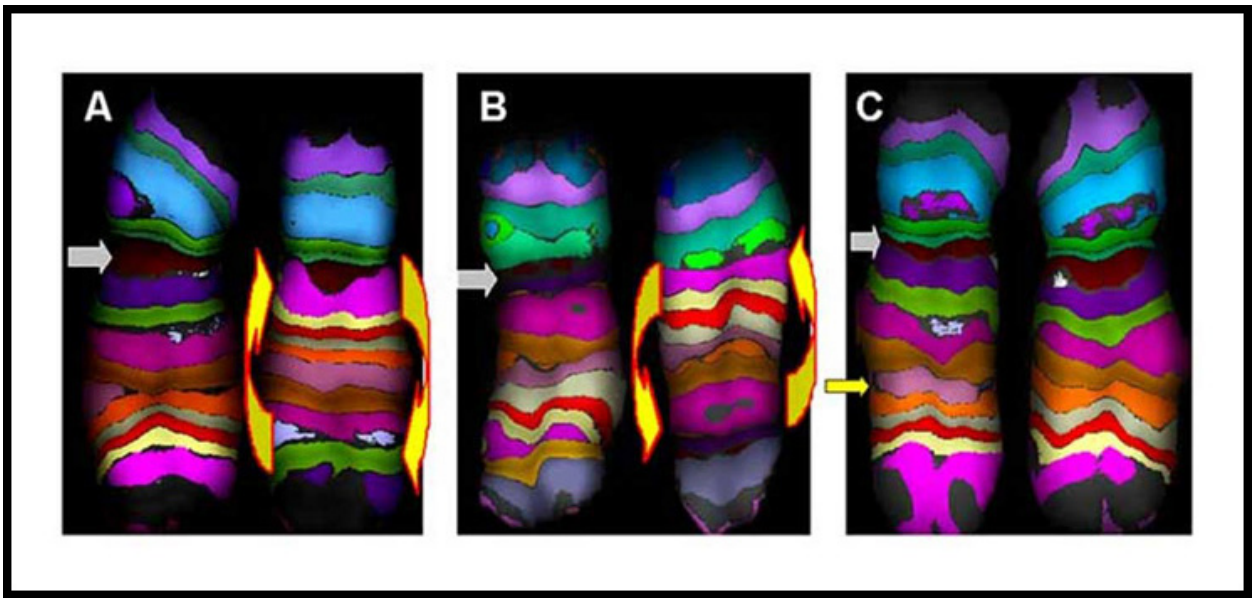


Figure 19. Intrachromosome rearrangements detected by multicolor chromosome banding. A and B are intrachromosal exchanges, C is normal (Brenner 2004).

However, the technique required to prepare the cells, the equipment needed, and scoring of the aberrations are expensive and is both time and labor intensive. This makes the technique useful in mechanistic studies but very difficult to apply to accidental exposures with multiple exposure types and conditions.

Nevertheless, techniques such as mFISH have been critical in understanding the location of genes, the types of rearrangements induced by radiation, and how the chromosomes interact to produce the observed structural changes. Such research is important and has resulted in an increased understanding of the microscopic distribution of the chromosomes in the nucleus and how chromosome location influences the frequency and type of chromosome aberrations (Plan et al. 2005). It was demonstrated that homologue chromosomes interact more frequently than others in the production of chromosome exchanges.

E. Micronuclei

The other form of chromosome damage that has been investigated in the DOE Program and used as a biomarker is the induction of micronuclei. Micronuclei are detected as small round pieces of chromatin material in the cytoplasm of interphase cells. After cell division these chromosome fragments will not be incorporated into the nucleus and are readily detected in the cytoplasm after the cell divides. The use of Cytochalasin B to prevent cytokinesis results in binucleated cells that make it possible to score for the production of micronuclei only in the cells that have undergone a cell division. Micronuclei are very easy to score and provide a quick measurement of radiation-induced chromosome damage. Extensive studies have been conducted to standardize the assay and make it very reproducible (Fenech et al. 1999). Because of this, recently micronuclei have been applied to many systems as a biomarker of radiation dose and exposure in human populations, as well as in other animals exposed to radiation. Additional research is being

conducted to develop methods to automate the scoring of micronuclei, which will make them very useful in biodosimetry.

A wide range of different human populations has been monitored for radiation-induced micronuclei. This includes such groups as hospital workers (Kryscio et al. 2001), nuclear power plant workers (Hadjidekova et al. 2003), uranium miners (Kryscio et al. 2001; Muller et al. 2004), and populations exposed to low dose rates of ionizing radiation (An & Kim 2002). Measuring chromosome aberrations in exposed populations using modern techniques provides a useful indication of radiation dose, even after complex exposures, which include both internally deposited materials and low dose-rate external exposures (Bauchinger et al. 2001). Many molecular epidemiological studies have been conducted outside the Program that help link the frequency of micronuclei to the induction of cancer. In these studies, which are not reviewed here, the risk for the induction of cancer is higher in individuals that have an elevated frequency of micronuclei.

F. Influence of Exposure Variables on Biomarkers

1. Dose

As is detailed throughout this book, biological alterations change as a function of dose. In the low dose region, many cellular changes do not increase as a function of dose but in fact have been demonstrated to decrease, as described earlier in this chapter as an adaptive response. For acute radiation exposure, other changes such as DNA damage and the formation of γH2AX foci are linear all the way down to the low dose region. As the dose increases, it seems that all changes increase as a function of dose. In the high dose region the measurement of biological damage provides a very useful biomarker of radiation exposure and dose. The types of biological changes described in this section that can provide biomarkers of exposure and dose range from the molecular, cellular, tissue and whole animals changes. Many of these can be automated and applied to large populations that may have been exposed to accidents, terrorist activities, or nuclear war and represent a useful area of research.

2. Dose rate

During the development of biomarkers, a number of studies measured not only the influence of dose on the endpoint, but also how these molecular and cellular endpoints changed as a function of dose rate. When radiation is delivered at a low dose rate, there was no increase in the frequency of γH2AX relative to the control level (Ishizaki et al. 2004). This demonstrates the importance of DNA repair of radiation damage as a function of exposure time. Thus, dose rate is very important in evaluating biomarkers.

DNA repair has been seen for both high- (Asaithamby et al. 2008) and low-LET radiation (Sutherland et al. 2002b; Sutherland et al. 2002a). The dose-rate effect has been demonstrated in most biological systems evaluated over the many years of research on the health effects of radiation (Brooks et al. 2009). There was a decrease in the frequency of micronuclei and an increase in apoptosis in cells exposed to low dose-rate radiation suggesting a protection as a function of dose-rate (Boreham et al. 2000). A marked decrease in the frequency of simple

exchanges involving two chromosomes was seen as the dose rate was decreased (Loucas et al. 2004b).

Studies of the cleanup workers at Chernobyl using chromosome aberration suggested that the dose reconstruction and recorded doses on these workers may have overestimated the true radiation doses estimated using chromosome aberrations detected with FISH techniques (Littlefield et al. 1998). An alternative interpretation of the data would suggest that radiation exposure at low dose rates, which many of these workers received, was less effective in producing biological damage than calculated from the current models used to correct for dose-rate effects. Thus, the measured doses may be accurate but the effective dose reduced. All these cellular and molecular observations support the need for a dose-rate-effectiveness factor (DREF) and the potential for a decrease in risk and effectiveness of low dose-rates relative to high dose-rate exposure (Brooks et al. 2009).

3. Radiation type

Use of chromosome painting techniques suggests that exposure to high-LET radiation can produce a unique and stable signature of radiation-induced damage (Brenner et al. 2001b). (Mitchell et al. 2004a; Mitchell et al. 2004c) suggested that such aberrations do not exist following exposure to low-LET radiation. Others expanded this research to investigate the production of chromosome aberrations in individuals that had body burdens of ^{239}Pu contamination from occupational or accidental exposures (Anderson et al. 2000; Anderson et al. 2003; Okladnikova et al. 2005). This research also suggested that alpha particles produced unique types of complex chromosome aberrations. The frequency of direct interaction and the deposition of energy from the alpha particles emitted by ^{239}Pu in the lymphocyte population are calculated to be limited. Thus, additional research is needed to determine if these aberrations are the result of the direct alpha exposure, bystander effects or to other factors in the environment of these workers.

Extensive research co-funded by NASA and DOE demonstrated that high Z (HZE) particles also produce unique biological changes. One of the most important changes can be measured as different types of chromosome damage. Using FISH techniques, complex chromosome aberrations were observed following HZE exposure. This complex damage suggests a useful biomarker of high-LET exposure (George et al. 2002; Hada et al. 2007; Kurpinski et al. 2009). Another chromosome change identified as being induced by HZE particles was chromatid type of exchanges in G_0 cells. These aberrations are not induced when G_0 cells are exposed to low-LET radiation. The frequency and type of aberrations that could be detected in G_2 cells using the premature chromosome condensation techniques were also found to be unique following exposure to HZE particles (Kawata et al. 2000).

Research identified other changes following high-LET radiation exposure that have different biological properties and may provide a biomarker of exposure to this type of radiation (Brenner et al. 2001b). Because of the dense ionizations produced along the track of a HZE particle, it was of interest to measure the induction of γH2AX foci. Individual cells and tissues were studied, and special imaging techniques (Costes et al. 2006) demonstrated the pathway of the traversal of HZE particles through the cell. The nature and size of the track indicated that a very large amount of energy was deposited in any cell that was traversed by an HZE particle

(Leatherbarrow et al. 2006). HZE exposures result in different biological effects, which can also act as biomarkers of this type of radiation exposure (Kurpinski et al. 2009).

4. Use of biomarkers in contaminated environments

Biomarkers identified from techniques developed in the Program have been applied to environmental and accidental radiation exposures and provide useful information in evaluating radiation hazards. The use of biomarkers to evaluate the radiation dose and damage at Chernobyl is an example of the importance of biomarker research funded by the Program. Early studies of the native bank voles in the zones around the accident suggested that the radiation dose and dose rate were very high (up to 10 mSv/day) and there may be extensive genetic damage induced in this population, and that this may have a negative impact on the animals that were living in the highly contaminated areas (Baker et al. 1996b; Baker et al. 1996a). The radiation doses and dose rates from both the external exposure and the internally deposited radioactive materials such as ^{90}Sr and $^{134, 137}$Cs were accurately measured in these rodent populations and related to the biological damage measured at the cell and molecular levels (Chesser et al. 2000; Chesser et al. 2001). The frequency of micronuclei and other genetic damage in the animals' bone marrow were evaluated and shown to have little increase relative to the levels seen in control populations (Rodgers & Baker 2000).

Because of concerns that the bank voles were radiation resistant, laboratory mice with known differences in radiation sensitivity—BALB, a radiation-sensitive strain, and C57Bl, a radiation-resistant strain—were taken into these highly contaminated zones and maintained in this radioactive environment for an extended period of time. Very limited indication of genetic damage occurred in either strain of mice under these chronic radiation exposure conditions (Rodgers & Baker 2000; Rodgers et al. 2001). The rodent populations at Chernobyl were followed for 3-5 years and there was no indication of an increase in mutation frequency or other genetic impact on them (Wickliffe et al. 2003b; Wickliffe et al. 2003a). Thus, no measurable genetic impact of the Chernobyl accident occurred in these animal populations, even though they were exposed to very large radiation doses delivered at a low dose rate. This suggests that dose rate is very significant in reducing the frequency of radiation induced damage.

This area has been made into a wildlife preserve. The ecological diversity, number of species, sex ratio, and other measures of the health of the ecosystem indicated that the ecological damage to this area is very minimal (Baker et al. 1996b; Baker & Chesser 2000).

Although there have been other extensive studies conducted in humans in other contaminated environments, the DOE Low Dose Radiation Research Program has not been involved in funding this epidemiological research. Nevertheless, the Program has provided the research, development and application of many of the techniques that were useful in this research. The spin-off value of the basic science conducted in the Program has had practical applications in the field of biodosimetry and biomarkers that reach far beyond the outlined goals of the program.

Major Points: Biomarkers

Many biomarkers are designed to detect radiation exposures in the high dose range.

- Research from the DOE Program has resulted in advances in the field of radiation biology that were applicable in development of biomarkers of exposure, dose, radiation sensitivity and disease.
- Chromosome damage provides one of the most reliable estimates of radiation dose. Modern techniques to stain and rapidly measure chromosome aberrations provide a rich source of mechanistic information on the interaction of radiation with cells.
- Molecular techniques have been developed to measure radiation-induced changes in DNA over a wide range of doses. The damage increases linearly with dose and thus provides a good biomarker of radiation exposure.
- The change in gene expression is potentially a great biomarker of radiation exposure since the techniques to measure these changes are very rapid, can be automated and applied to evaluate large populations exposed to ionizing radiation.
- Exposure variables (dose, dose-rate and radiation type) all have a marked influence on the response of biomarkers and must be considered in their application.
- Biomarkers from techniques developed in the Program have been applied to environmental and accidental radiation exposures and provided useful information in evaluating radiation hazards.

Chapter 6

Mechanisms of Action

I. Need for Mechanistic Studies

When the DOE Low Dose Radiation Research Program issued its first call for proposals in 1999, many scientists suggested that they could not measure biological changes in the dose range of interest, <0.1 Gy (10 rads). Dr. Marvin Frazier, the Program's scientific director, replied that in that case, they need not apply. This was good advice, and many new techniques and technologies were developed and applied by Program-supported scientists that made it possible to detect previously undetectable radiation-induced biological changes in the low dose region. These new observations suggested the need for major paradigm shifts in the field of radiation biology.

Before the Program, the proposed mechanisms of action for radiation in the low dose region were easy to understand: radiation produced DNA damage, mutations, chromosome aberrations, and cancer. The induction of DNA damage had been observed and measured only in the high dose region and damage increased linearly with radiation dose. Mutations were observed in many different biological systems ranging from single-cell organisms to plants and mammalian cells in culture.

At high dose rates, mutations also increased linearly with radiation dose. Chromosome aberrations increased in all biological systems exposed to radiation in a well-defined function of dose, which at low doses seemed to be linear. Carefully conducted studies demonstrated that after high dose, high-dose-rate radiation exposure, cancer frequency increased in experimental animals and humans. As the dose rate decreased, cancer frequency decreased markedly in animal models. However, in the low dose region, the epidemiological tools available were not sensitive enough to detect increases in cancer frequency in humans.

The uncertainty in the human data generated was so great that little could be said about the shape of the dose-response curve or if any increase in cancer frequency occurred in the low dose region. However, together these observations suggested that radiation caused damage in the genetic material, the amount of genetic damage was linearly related to the radiation dose, and the genetic damage was the primary cause of radiation-induced cancer. Therefore, the radiation dose was directly and linearly related to cancer induction. This was a useful mechanistic approach for high dose and high-dose-rate radiation exposure.

As described in the previous chapters, technologies and techniques now exist and have been used to evaluate the radiation response in the low dose region, and scientific observations in the low dose region suggest that radiation risk needs reevaluation. Scientific committees such as the National Academy BEIR VII committee and ICRP acknowledged these phenomena but suggested it was difficult to determine how the new data could be used to impact the understanding of radiation risk in the low dose region. When the BEIR VII report "Health Risks from Exposure to Low Levels of Ionizing Radiation" and the ICRP 103 were written in 2006, it

was suggested that the observations of genomic instability, adaptive responses, and bystander effects were not adequately supported by data on molecular mechanisms to be useful in risk assessment (NRC 2006; Valentin 2007).

The BEIR VII committee suggested "…until the molecular mechanisms responsible for genomic instability and its relationship to carcinogenesis are understood, extrapolation of the limited dose-response data for genomic instability to radiation-induced cancers in the low-dose range <100 mGy is not warranted." For adaptive responses the committee said, "Thus, it is concluded that any useful extrapolations for dose-response relationships in humans cannot be made from the adaptive responses observed in human lymphocytes or other mammalian cellular systems. Therefore, at present, the assumption that any stimulatory effects of low doses of ionizing radiation substantially reduce long-term deleterious radiation effects in humans is unwarranted." Finally, for bystander effects, "Until molecular mechanisms of the bystander effects are elucidated, especially as related to an intact organism, and until reproducible bystander effects (changes in gene and protein expression, changes in ROS status of the cells, changes in many physiological pathways) one electron track traverses the nucleus, a bystander effect of low dose, low-LET radiation that might result in modification of the dose-response should not be assumed."

At the time of the BEIR VII report, the lack of mechanistic data suggested that the new science could not be used in risk estimates. However, since then, the Program has generated additional mechanistic data on each of these responses. This chapter summarizes the mechanistic data and provides a source of information for future research. To put all these data together using a systems approach will require additional research and funding, but it is essential to be able to use the information produced to date to understand risk, the mechanisms involved, and the shape of the dose-response relationships in the low dose region.

It is imperative to examine the new radiation biology produced by the Program to summarize the data; describe the cell and molecular pathways triggered and altered by low dose radiation exposure; review the important genes, proteins, chemicals and pathways involved; and suggest potential approaches to use these mechanistic data to evaluate their impact on the shape of the dose-response relationship in the low dose region.

This chapter is intended to summarize the mechanistic data from the Program and draw conclusions about its potential significance. These conclusions in this book are the view point of the author and may not relect the opinions of the scientists involved in the program on the DOE low dose program. In light of the new complicated and interactive responses observed following the exposure to low doses of ionizing radiation, it is no longer acceptable to suggest that there is a single mechanism of action.

As mechanistic data continue to be developed, it is essential to use systems biology approaches to develop models that describe important pathways, and provide relationships and weights for each pathway and information on the ultimate shape of the dose-response relationships in the low dose region. Such evaluations will help to understand and evaluate radiation risk and incorporate these mechanistic data into the calculation of risk factors. This approach will require extensive interactions between research groups, a serious commitment of resources, methods to accumulate

and share data, and a commitment to developing and applying the models developed. Early models that pave the way for these efforts have been developed and suggested approaches for systems approaches that can develop more mechanistic models will be discussed in Chapter 7.

II. Dose and Energy Deposition

When ionizing radiation deposits its energy in biological systems, molecules are altered. These initial alterations are the basis for all subsequent biological responses. It is critical to be able to relate the proper energy metric to the subsequent biological response as the basis for developing models to describe these responses (Brooks 2005). The deposited energy can be described in several ways. A report was recently developed by the (ICRU 2011) concludes that the energy metric is very important in conducting and understanding radiation biological experiments. A major point is that the energy distribution and deposition pattern in cells, tissues, and organisms is very non-uniform especially in the low dose region. It is important to understand how this non-uniform deposition of energy influences the biological outcome. Microbeam studies have been important in evaluating the influence of dose-distribution on biological effects and suggest that the radiation response target is much larger than that of energy deposition (Braby & Ford 2004).

The primary descriptor for deposition of energy in a tissue has been energy/unit of mass or dose. The development and use of dose as the most important metric in radiation biology is widely accepted. The universal use of dose in radiation biology suggests that the concentration of the interactions and ionizations produced by the radiation plays a central role in the observed biological response. This makes dose rate important because the concentration at any time also plays a central role in radiation biology in the types of responses and pathways activated. Most of radiation biology, medicine, and risk assessment has been based on these two metrics of radiation exposure.

However, (Bond et al. 2005) suggested that, if the LNT model is important, the total number of ionizations produced (energy deposited in the system) should be the biological response metric. If this were the case, the radiation-induced biological effects would relate not to dose but to the total energy deposited in the system of interest.

(Bond et al. 2005) also suggested that total energy deposited in the biological system be used as the basis for setting radiation protection. Using total energy in the system rather than energy concentration (dose) as a metric for describing radiation response would have major impact on radiation biology.

First, it can be demonstrated that for any biological system there is an amount of energy deposited below which it is not possible to detect a biological response. This threshold or energy barrier must be exceeded to detect a significant biological response (Brooks et al. 2006a) (Brooks et al. 2000; Brooks et al. 2006a).

Second, if energy is used as the metric for evaluating biological responses it becomes obvious that very large amounts of radiation (energy) are required to produce a detectable excess cancer above the high background level of spontaneous cancer (Brooks et al. 2007). Evaluation of the atomic bomb data using this concept demonstrated that if this large amount of energy were

deposited in a small population it would be lethal. However, because the energy was distributed over a very large population, an increase in responses cannot be detected. This provides a very different view of radiation exposure and risk than when dose is used as the metric for radiation-induced cancer.

The current concept is that every ionization increases the risk for the induction of cancer (NRC 2006). Thus, low doses of radiation, delivered to a large population, increase the calculated risk. Using total energy deposited rather than radiation dose as the metric for radiation exposure and risk provides a very different perception of the risk associated with low doses of radiation. It takes a very large amount of radiation (Energy in Joules) to produce excess cancer. Such information should be considered in communicating with the public, regulators, and those concerned with accidental radiation exposure or terrorist events.

III. Biological Mechanisms of Low Dose Responses

A. Radiation-Induced Changes in DNA

Years of radiation biology research have demonstrated that DNA damage and repair are the earliest and most important changes induced by ionizing radiation, as it plays an important role in the induction of mutations and cancer. The huge database from this research has been used as the mechanistic basis for development of the hit theory, the biophysical models used to predict the induction of cancer, and the LNT (NRC 2006) and to describe the relationship between radiation dose and cancer development risk. These data remain a critical part of radiation biology.

Because of the extensive database in place at the time DOE Program began, the Program's focus has not been on the role of DNA damage on cancer risk, but on the differences in DNA damage induced by radiation and DNA damage from other sources, including the endogenous DNA damage that is present in large amounts. The other focus of the Program has been on the role of DNA alterations as a signaling mechanism to alter gene and protein expression, metabolic pathways, and cell function. The information developed has been beneficial in understanding the responses seen in the very low dose region where the frequency of mutations is very low and the major impact of the radiation-induced DNA alterations seems to be related to other important biological changes.

B. Differences between Radiation-Induced and Spontaneous DNA Damage

Program-funded research has made progress in identifying the differences between radiation-induced damage and that produced by endogenous factors, as reviewed in Chapter 5. The mechanisms involved in the production and repair of clustered DNA lesions has been reviewed (Prise et al. 2001) and remains an area of continuing research effort. Early in the Program, it was suggested that clustered lesions were unique to radiation and that such damage would be very useful as a biomarker of radiation exposure (Sutherland et al. 2001b). (Pinto et al. 2005) suggested that the clustered lesions had unique structure and may be very difficult to repair. These lesions which have a different spacial distribution (Rydberg et al. 1994) were induced more frequently by high-LET radiation than by low-LET X-rays or gamma rays (Paap et al. 2008). Studies on the repair of these lesions using human abasic endonuclease (Ape1) suggested

that cellular responses to complex damage may be carried out by multiple processing mechanisms, and that the more complex nature of high-LET-induced damage would have more serious consequences in terms of risk and cellular damage than simple single- or double-strand DNA breakage, which is consistent with other data on high-LET radiation. However, further research determined that there were low levels of clustered lesions induced by endogenous oxidative damage in the normal cells, and that the lesions may not be unique to ionizing radiation (Sutherland et al. 2003c). These observations were extended to human skin and further studied in human skin models (Bennett et al. 2004; Bennett et al. 2005).

Such research suggests that clustered lesions are part of normal human biology and may be produced by endogenous and other environmental insults in addition to ionizing radiation (Bennett et al. 2005). In CHO cells it was demonstrated that increased fgp protein lowered clustered damage and the frequency of Hprt mutations (Paul et al. 2006). The induction and repair of these lesions by both high and low-LET radiation and their role in radiation risk remains an important area for future research.

C. Signaling Molecules

1. DNA alterations as signaling molecules

Radiation-induced DNA damage is detected by cells and results at the start of a number of cellular events, many of which are involved in the repair of this damage. Double-strand breaks are thought to be the most important primary lesion induced by ionizing radiation (Jeggo 1998) involved in cell killing (Olive 1998). The repair of DNA damage has been reviewed and is available as a flow chart at www.abcam.com/nuclearsignal, which was produced in collaboration with James Haber and Farokh Dotiwala of Brandeis University. The genes and proteins involved in the different types of DNA repair are well defined in this flow chart.

2. The role of ATM in signaling

An important breakthrough in the study of DNA damage and repair was the observation that individuals with the disease ataxia telangiectasia have a deficiency in DNA repair. People with this disease have a mutated gene that produces an altered protein called ataxia telangiectasia mutated protein (ATM). The cDNA for ATM was first characterized by (Zhang et al. 1997). This gene has been carefully studied and the protein has been isolated and characterized (Lavin 1999). The normal protein associated with the mutated gene was able to correct the phenotype of cells that had the mutated ATM gene (Lavin 1999). (Lavin et al. 2005) also determined that DNA damage altered ATM signaling in a way that resulted in genomic instability. These observations suggest a link between DNA damage, ATM, genomic instability, and the induction of cancer. Studies of cells, tissues, and organisms with this mutated gene and protein have provided an essential tool for study of radiation-induced DNA damage and repair. This protein, kinase ATM, is the primary transducer of cellular responses initiated by DNA damage caused by ionizing radiation.

Phosphorylation has been shown to be very important in the repair of DNA lesions (Whalen et al. 2008). The phosphate forms of important proteins such as γH2AX are found to localize at the site of DNA DSBs (Rogakou et al. 1998) and have been strongly correlated with and sometimes

used as markers of the frequency and repair of DNA breaks (Rothkamm & Lobrich 2003). These lesions have also been suggested as potential markers of cell killing induced by drugs that produce DNA DSBs (Banath & Olive 2003).

It has been shown that ATM, HDM2, p53, and DNA-PK are all involved in H2AX phosphorylation at the site of radiation-induced DNA DSBs (de Toledo et al. 2000; Stiff et al. 2004). It was also demonstrated that phosphorylated p53 directly binds to radiation-induced DNA breaks (Al Rashid et al. 2005) and that p53 is involved in a global regulation of genomic repair genes (Amundson et al. 2002). As a function of radiation type, phosphorylation is very dependent on radiation quality, further emphasizing that the response to radiation is unique as a function of radiation type, and correcting for the increased effectiveness of high LET radiation by a simple number may depend on the endpoint and the biological processes activated by different radiation types (Whalen et al. 2008).

However, these DNA repair foci also form during many normal cellular processes, so care must be taken to control many biological variables when using foci as measures of the amount of DNA damage induced by ionizing radiation (Tanaka et al. 2007). Studies have been conducted to compare the disappearance of DNA DSBs and the loss of γH2AX foci. The loss or repair rates between these two endpoints do not have a one-to-one correlation. This suggests that they are related but not identical (Kato et al. 2008). Like most biological responses to radiation, there is a difference in individual sensitivity to the induction of γH2AX, showing that genetic background is of prime importance in radiation responses (Hamasaki et al. 2007).

Cells and organisms that are heterozygous for ATM have been shown to be lacking in DNA repair as measured by γH2AX (Kato et al. 2006) and to be sensitive to radiation-induced cell killing (Kuhne et al. 2004), oncogenesis (Smilenov et al. 2001; Hall et al. 2005), and formation of cataracts (Worgul et al. 2005). Studies of the role of ATM on the change in the responses to low doses of radiation from sensitivity to the induction of low-dose radiation resistance suggest that this process is independent of modification of ATM at the ATM ser1981 site (Krueger et al. 2007b).

It has been demonstrated that even a transient inhibition of ATM kinase is sufficient to enhance radiation-induced cell killing (Rainey et al. 2008) and that ATM knockout heterozygous mice display a marked adaptive response for the induction of mutations (Day et al. 2007a). Both observations support the hypothesis that ATM plays an important role in both mutation and in cell sensitivity to ionizing radiation. However, other studies on mice with haploinsufficiency suggested that this condition does not affect the frequency of mutations in solid mouse tissues (Connolly et al. 2006). Haploinsufficiency for ATM and Mrad9 can increase the effects of radiation in the production of cataracts (Kleiman et al. 2007). This condition also changes some of the early events to initiate cell signaling and changes in the function of the cells (Smilenov et al. 2005). The signaling cascade induced by induction and repair of DNA damage has also been shown to influence a number of measurable cellular events such as cell cycle progression, gene activation and expression, changes in ROS status of the cells, senescence, and apoptosis (Smilenov et al. 2005).

3. The interactions between ATM and p53

Radiation exposure can be linked to ATM activity and p53 is downstream from ATM. Thus, the data have demonstrated that radiation exposure to low doses causes a marked change in gene expression as well as a complex cascade of signaling events. Biological pathways in this cascade have been studied and many involve the p53 gene (Amundson et al. 2005), the "guardian of the genome," and it is involved many repair processes (Amundson et al. 2002) as well as in the induction of cell death and apoptosis (Slee et al. 2004). The induction of apoptosis is the most conserved function of p53 and seems to be vital for tumor suppression (Slee et al. 2004). However, when this gene is mutated it can become the "fallen angel" and result in many biological problems and diseases. A well-defined database (http://www-p53.iarc.fr/) has been developed that summarizes the involvement of mutated TP53 genes in the induction of human cancers. The mutational status of the TP53 gene is critically important in influencing the tumor phenotype (Deppert 2007).

The importance of p53 in radiation-induced effects is well established regarding changes in gene expression, radiation damage, control of chromosome instability (Schwartz et al. 2003), and cell survival (Schafer et al. 2002; Amundson et al. 2008). The individual variability in p53 and CDKN1A has also been linked to radiation sensitivity (Alsbeih et al. 2007). The expression of p53 shows that it is a key gene in controlling chromosome integrity (Honma et al. 2000), chromosome instability (Schwartz et al. 2003), and the genomic stability of cells (Perez-Losada et al. 2005). Such studies show that p53 is at the center of a complex web of incoming stress signals and outgoing effector pathways. Understanding these pathways and signals provides links between p53, environmental exposures (Medina et al. 2002), stress (Amundson et al. 2005; Horn & Vousden 2007), genomic instability (Mao et al. 2005), and cancer induction (Yang et al. 2000b; Yang et al. 2000c) as well as many other diseases.

It is important to study the relationships between radiation-induced changes in p53 and changes in other genes and proteins to understand how early genetic events are related to later changes in cells and tissues. This provides insight into how these early changes can be related to animal and human models of cancer. An example of this is found in a study by (Williams et al. 2008b) where the genotype and radiosensitivity of 39 human tumors were evaluated, and it was determined that the normal and mutant TP53 status of the tumors was critical in determining both the radiosensitivity of the cells and histology-dependent variations in radiosenstivity of the cancers. This research suggested that radiosensitivity can predict responses of human tumors to radiotherapy protocols.

Changes in metabolic pathways associated with p53 are important and provide some of the early biological linkages to diseases as well as play a role in the induction of bystander effects (Prise et al. 2006a; Burdak-Rothkamm et al. 2007; Burdak-Rothkamm et al. 2008). Program research has also identified many chemicals and factors that modify the radiation-induced changes in these pathways.

PubMed has about 3600 citations per year on p53 research (Deppert 2007); therefore, the database cannot be covered here detail. However, large databases are available that summarize the knowledge about the role of p53 (http://p53.bii.a-star.edu.sg) in radiation response and mechanisms of cancer induction (Lim et al. 2007; Amundson et al. 2008).

p53 activity is modulated by MDM2, and the dose of radiation delivered is important in regulating this interaction. At low doses, there is little induction of p53 or MDM2. As dose increases, the level of p53 peaks followed by a down-regulation as MDM2 peaks. This cycle repeats itself depending on the radiation dose. At low doses, there are few cycles. As dose increases, the magnitude of the peaks remains constant but the peak replications increase. Following very high doses, the interaction continues for a prolonged period of time (Tyson 2004). This critical interaction shows a threshold below which no measurable changes occur. Using this approach, it has been possible to show that p53 signaling and the interaction with MDM2 antagonists are modified in some cancer types. This may have implications and applications in potential cancer therapy (Tovar et al. 2006).

D. Summary of Biological Response Mechanisms

Once the cascade of responses has been initiated by the radiation-induced DNA damage many questions remain with major areas of research as follows:

- Many genes are activated by low doses of ionizing radiation. These genes modify many chemical factors and metabolic pathways within the cell that are responsive to low doses of radiation. These pathways have been studied in the Program, and it has been determined that they influence the responses in cells both with and without energy deposited in them. These effects are important in the subsequent development of biological changes measured in a wide range of systems, because they may modify response that either decreases or increases radiation-related risk. For many of these pathways, it is not possible to determine which way (beneficial or detrimental) these influences will go. However, the weight of the evidence currently favors the induction of protective effects induced by low doses of ionizing radiation.
- Considerable research has been done on the chemicals and factors involved in transmitting the signals from cells that have energy deposited in them to other cells. This transmission is manifested by the extensive cell-cell, cell/matrix, and cell tissue interactions. Research on chemicals involved in signaling is one of the Program's major areas. It is essential to identify the signals, the targets, and the responses generated.
- The molecular changes result in cellular changes critical to the total response. Such changes depend on interactions between cells and can result in marked changes at the tissue and organ level. New techniques and methods in proteomics and metabolomics are being developed to evaluate all these changes. With these techniques, it has become possible to understand more of the mechanistic basis of the radiation responses to low doses of radiation. Such research will help determine the path forward to consider the mechanisms involved in the radiation-related changes and provide important data for modeling the responses induced by exposure to radiation in the low dose regions.
- It is important to review the influence of all these mechanisms on inducing genomic instability, bystander effects, and adaptive responsive, as suggested in early observations. Research has demonstrated that these responses are linked by common cellular mechanisms. Because of the large amount of data generated in each area it is not possible to review it completely. This book focuses on the Program-generated publications and research in these areas. It is important that all these factors be considered to construct

models that not only describe cellular and molecular events but use systems approaches to identify how such information can be used to evaluate radiation risks.

1. Transmission of the signals to other cells

Chapter 5 covered the experimental studies that established that bystander effects are a well-defined response to low doses of radiation. The bystander effect is in reality a measure of the communication that occurs within biological systems. Studies have been conducted to determine how radiation-induced changes can be communicated as cell-cell, cell/matrix, and cell/tissue interactions. The bystander effect studies demonstrated that the cells that have energy deposited in them communicated the changes induced by the radiation to the neighboring cells (Shao et al. 2003a; Laiakis & Morgan 2005; Sandfort et al. 2007; Shao et al. 2007).

Such responses as DNA damage and homologous recombinational repair influence the induction of bystander responses (Nagasawa et al. 2005). In cells that are defective in homologous recombination, there is no bystander effect seen for the induction of SCE or chromosomal aberrations (Nagasawa et al. 2008). The induction of cell killing (Schettino et al. 2003; Schettino et al. 2005; Baskar et al. 2007) and apoptosis (Vit & Rosselli 2003; Vines et al. 2009) in bystander cells has been well documented, as has the induction of micronuclei (Konopacka & Rzeszowska-Wolny 2006). Bystander effects could be produced by either high or low LET radiation using microbeam technology (Baskar et al. 2008). Also, (Ponnaiya et al. 2007) determined that there are changes in gene expression in cells that are not directly "hit" by ionizing radiation. As outlined in Chapter 5 cell transformation was one of the first and most important observations made for the induction of a biological effect in cells without direct energy deposition in them (Mitchell et al. 2004c). As research has continued, the term "bystander effects" has been the subject of extensive research to be defined in more mechanistic terms (Hei et al. 2008). This communication can alter biological responses to radiation in ways that could be considered both protective (adaptive responses) and harmful (genomic instability) to the body.

After the discovery of bystander effects induced by low doses of alpha particles (Nagasawa & Little 1992) using the induction of SCE as an endpoint, there were many discussions as to how and if these responses were a general biological phenomenon. With the development of the microbeam by Program investigators, it was possible to know which cells had energy deposited in them and to measure the responses in these cells as well as in cells with no energy in them. It is necessary to identify both the "hit" cells and the "non-hit" cells and to determine the pathways involved in the cell-cell communication. Finally, the chemicals and factors released must be identified, and their interaction with the target cells characterized. Experimental techniques must be developed that can determine how the-bystander cells are modified and provide measurements of the factors released and the responses induced (Hill et al. 2006; Pyke et al. 2006). These studies demonstrated that bystander responses were very general and have now become well accepted. Research is being conducted to address these scientific questions and determine the mechanisms involved in the cell-cell communication.

It has been known that gap junctions play an important role in cell-cell communication in many cell types and that the protein connexin 43 was an important molecule in this communication. This information was used early in the Program to determine that direct cell-cell contact and the presence of gap junctions were critical for the cell-cell communication induced by low doses of

high-LET ionizing radiation (Glover et al. 2003). Studies also demonstrated that connexin 43 was sensitive to low doses of ionizing radiation and other environmental stresses. The cell-cell communication induced by low doses of radiation can be modified by blocking connexin 43 using Lindane. Bystander effects were eliminated by this treatment (Azzam et al. 2003a). Such studies demonstrated that gap junctions and direct cell-cell contact is essential in some types of bystander effects.

The molecules released and thought to be important in the induction of cell-cell communication and bystander effects following low dose radiation exposures have been an important area of research in the Program. It was thought that the molecules should be small and be able to travel quickly between cells to account for the rapid responses observed in the cells that did not have energy deposited in them.

An early candidate that was critical to the bystander effects was nitric oxide (NO). This molecule has a long enough life (>7 minutes(Hakim et al. 1996). Half-life of nitric oxide in aqueous solutions with and without haemoglobin) to move some distance from the cell exposed to the ionizing radiation (Belyakov et al. 2005), is involved in oxidative metabolism (Azzam et al. 2002; Ridnour et al. 2005), and has been linked to transforming growth factor beta 1 (TGFβ1) in the induction of bystander effects in glioma cells (Shao et al. 2008a). This molecule and oxidative metabolism seemed to also provide mechanistic links between gap junctions, bystander effects, and adaptive responses (Azzam et al. 2003b). (Ridnour et al. 2005) showed that nitric oxide treatment can induce resistance to oxidative stress induced by hydrogen peroxide, apparently through a glutamate cysteine ligase activity-dependent process. This observation supports the other low dose responses, where it has also been shown that low doses of radiation activate genes involved in the production of MnSOD (Guo et al. 2003) and glutathione, which are both involved in the protective process against damage from radiation induced free radicals and oxidative stress (Guo et al. 2003). Many other genes involved in adaptive responses have been identified that are also involved in protective processes (Tomascik-Cheeseman et al. 2004; Chaudhry 2006). It has been suggested that NO radicals choreograph the adaptive response and provide a strong link between the induction of adaptive responses and bystander effects (Matsumoto et al. 2007). Such research provides examples of how bystander effects could result in a protective response, as opposed to the induction of potentially harmful changes such as the micronuclei and other genotoxic responses observed in bystander cells (Azzam et al. 2002).

(Shao et al. 2006) observed that calcium flux was modulated by low doses of radiation and was an important change induced in bystander cells. This flux was postulated to be critical to the induction of bystander effects in glioma cells and fibroblasts. The molecular pathways activated by the calcium seemed to involve the MAP kinase signaling pathways (Lyng et al. 2006) and was shown to be involved in the induction of bystander responses in cells without energy deposition. Additional studies using glioma cells helped define the role of other signaling factors induced by ionizing radiation (Shao et al. 2008b).

In media transfer experiments it was shown that materials released from the cells produced paracrine-signaling pathways involved in cell transformation in response to low doses of low-LET radiation (Weber et al. 2005). Many other media transfer experiments demonstrated bystander effects induced in cells exposed to conditioned media (Mothersill et al. 2006; Maguire

et al. 2007). These studies were done in JB6 cells that are easily transformed by a number of factors. Other studies showed that autocrine signaling was also important in cell-cell communication and that epidermal growth factor (EGF) was involved in this signaling (Chen et al. 2004b).

Research to identify the signaling pathways suggested that many complex pathways exist and play an important role in cell-cell communication. For example, both protein kinase C (Baskar et al. 2008) and cyclooxykgenase-2 (Zhu et al. 2005) pathways were demonstrated to be important in cell signaling resulting in biological changes in cells that did not have direct energy deposited in them. The magnitude of these indirect changes could be increased following exposure to either alpha particles (Han et al. 2007) or low LET (Zhou et al. 2008) radiation exposures by simply changing the NaCl concentration in the media. Such studies emphasize the need for control and reproducibility of research in studies of cell-cell communication and the potential for artifacts to be introduced in the data sets.

Additional research using proteomic and other biochemical approaches linked the calcium changes to phosphorylation-dependent calmodulin complex in mammalian cells, both of which are important in cell-cell communication (Jang et al. 2007). It may be that this is an additional pathway associated with bystander responses. As is often the case for these molecular and cellular responses, the same factors do not seem to apply for different types of radiation exposure. Heavy ion exposure, such as that encountered in space, failed to activate the calcium pathways and/or modify early calcium flux (Du et al. 2008).

E. Metabolic Pathways

The radiation-induced pathways are interlinked, so it is not possible to discuss them independently. They have been broken down here and discussed by the chemicals and pathways involved. As the signaling pathway continues through many different critical locations, research has demonstrated that alterations from a wide range of factors can modify the signaling and the biological outcome. Elaborate methods have been designed to link all the various interactions with radiation exposure using several different techniques, but those will not be reviewed here.

1. TGFβ

Early in the Program, (Barcellos-Hoff & Brooks 2001) recognized that transforming growth factor beta (TGFβ) was important in the expression and modification of direct and bystander-induced radiation-induced damage both *in vitro* and *in vivo*. Important relationships were established between the induction of DNA damage, TGFβ, and different types of cellular damage (Ewan et al. 2002). These relationships were extended from the role of TGFβ at the single-cell level to TGFβ interaction at the tissue level. (Barcellos-Hoff 2005a) demonstrated that this compound played an important role in orchestrating the interactions and outcome of radiation with tissues. Further research showed that inhibition of TGFβ blocked ATM activity to genotoxic stress (Kirshner et al. 2006). This again supported a direct link between DNA damage and TGFβ. Other pathways were also demonstrated to play an important role in signaling and controlling DNA repair, including the signaling from epidermal growth factor receptor (EGFR)(Rodemann et al. 2007).

(Jobling et al. 2006) established that the ROS status of cells and tissues were directly linked to both radiation exposure and the treatment with TGFβ. Changes in ROS status have also been linked to cell-cell communication and the bystander effect. TGFβ was shown to modify NO activity as a possible mechanism for its role in cell-cell communication (Shao et al. 2008b). NO also played a major role as a signaling molecule during the induction of bystander effects in gleomia cells as they communicated with fibroblasts (Shao et al. 2008b). Many of these changes in cell signaling and the ROS status of cells have been shown to modify the response to genotoxic agents including radiation.

Other important cellular changes directly related to TGFβ may play a role in cancer development. (Andarawewa et al. 2007b) showed that treatment with TGFβ was critical in the transition of epithelial cells to mesenchymal cells. Such a transition could influence the progression of normal epithelial cells to cancer cells. TGFβ was also implicated in modification of the immune response. The regulation of immunological responses during the development of skin cancer suggested an important role of this compound in the carcinogenesis process (Glick et al. 2008). (Ewart-Toland et al. 2004) demonstrated that a gain of function of TGFβ1 polymorphism plays a role in late stage prostate cancer and may act as a potential biomarker for the progression of this disease. Because of these observations, it was suggested that TGFβ could be used in conjunction with radiotherapy. This hypothesis is being tested (Andarawewa et al. 2007a).

The central role of TGFβ in radiation responses has thus been well established. Additional research in the low dose region to determine the impact of this compound on cancer risk is essential.

2. NF-κB

NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of DNA. It is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, and free radicals produced by ionizing and ultraviolet irradiation. NF-κB is important in maintaining genomic stability and is modified in cells that display genomic instability. Incorrect regulation of NF-κB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development.

NF-κB is important in the biological response to ionizing radiation. It is directly linked to the signaling pathway from radiation-induced DNA damage through its interactions with ATM. This interaction has been thought to be important in generating radiation-sensitive cells (Ahmed & Li 2007). The ErbB2 pathway is also involved in radiation-induced activation of NF-κB (Guo et al. 2004). Radiation-induced pathways such as NF-κB that modify radiation response are an important part of the intercellular signaling triggered by low doses of radiation that play a role in split-dose repair.

When radiation exposures are split into multiple fractions with a time period between each of the dose fractions, cell survival increases. This is thought to be made possible by repair of damage

produced by the first fraction before it can interact with that produced by later fractions. The interaction between NF-κB and other molecules involved in cell signaling plays an important role in repair of damage between the fractionated doses that is critical to cell survival (Mendonca et al. 2007).

Studies have defined the signaling pathways involved in NF-κB using a variety of different techniques. Low doses of gamma rays activated NF-κB in the bone marrow of mice (Rithidech et al. 2005). Knock-out and transgenic mouse models were used to define the role of NF-κB in cancer induction (Gerondakis et al. 2006). Such studies provide mechanistic pathways to be integrated into the total radiation responses. NF-κB is involved in cross talk critical in sensitizing cells to cell killing by ionizing radiation (Bubici et al. 2006). It has also been shown that NF-κB inhibits mitogen-activated protein kinase signaling in radio-resistant cancer cells, which modifies cell proliferation and increases survival. Such activity also suggests an important role in decreasing radiation-related cell killing (Ahmed et al. 2006). These cytotoxic responses seem to be mediated through interactions with mitochondria. This interaction with the mitochondria and the ROS status of the cell plays an important role in signaling for the induction and promotion of radiation-induced bystander effects (Zhou et al. 2008).

Research suggests that the potential for increasing cell killing through NF-κB is involved in the induction of the adaptive response (Ahmed & Li 2008). The cell killing decreases the frequency of abnormal cells as a function of radiation dose and results in adaptive responses. As these studies have provided a more mechanistic understanding of NFκB's role in cell killing, it has been suggested that NF-κB could play an important role in cancer therapy (Ahmed & Li 2007).

The role of NF-κB in the induction of genomic instability is also a critical factor and requires additional research. Maintaining the stability of the genome is critical both in cells that are directly hit by radiation and in cells where the signals from hit cells induce changes. Research by (Moore et al. 2005) indicated that in addition to NF-κB, tumor necrosis factor alpha (TNFα) is modified in cells that have energy directly deposited in them from alpha particles but is not altered in bystander cells. It has been suggested that modification of oxidative stress in cell signaling and the importance of the oxidative status of the cells are critical in maintaining and modulating genomic instability induced by TNFα. Modification of these factors has been reported to increase radiation-induced genomic instability (Natarajan et al. 2007).

The concern about atomic war and terrorism has resulted in extensive research on the role of chemicals in modifying radiation responses to protect cells and tissues from ionizing radiation. The Program has not funded extensive research in this area. However, as adaptive response became a recognized result of exposure to low doses of ionizing radiation, it was important to determine the mechanisms involved in this adaptive response and to evaluate some of the chemicals that may protect against radiation.

3. WR-1065

Research in the Program determined that changes in the levels of reactive oxygen and Mn Superoxide dismutase (MnSOD) protected against radiation damage and were involved in

adaptive response. It was thus important to understand how other chemicals could modify and protect against radiation-induced damage.

A few projects were funded to evaluate the role of the radioprotection product amifostine (WR-1065), one of the few compounds been used in clinics to modify radiation responses. (Murley et al. 2002) demonstrated that mouse sarcoma cells could be protected by the enhancement of MnSOD gene expression when exposed to the active metabolite of WR 1065. Oxidative status of the cells and cytoprotection was also modified by both amifostine and TNFα through changes in levels of manganese superoxide dismutase (SOD2) (Murley et al. 2007). This produced radioprotection through changes in the free thiol form of aminfostine (Natarajan et al. 2007), which had been recognized as being important in activating NFκB gene expression. Such research provides one potential mechanism for the radioprotective action of WR 1065 (Grdina et al. 2002; Kataoka et al. 2002).

The active metabolite of amifostine was recently shown to mitigate radiation-induced genomic instability (Dziegielewski et al. 2008). Because genomic instability has been suggested to play a critical role in the induction of cancer, these studies provide potential pathways for modification of the cancer response and link these molecular studies to cancer risk.

4. Clusterin

Clusterins are stress-inducible polypeptides that play an important role in cell survival, proliferation, and apoptosis. Through such actions, they have been implicated in the induction of multiple organ dysfunction following high doses of radiation (Araki et al. 2005). Nuclear clusterin has been synthesized, and its function as an inducer of cell killing defined (Leskov et al. 2003). However, clusterin's activity is modified by its cellular location because clusterin(s) can be located and processed either in the nucleus or can be secreted into the cytoplasm and outside the cell. Clusterin expression has been shown to be induced by low dose ionizing radiation. It can act to either kill cells or improve the survival depending on its type and cellular location (Leskov et al. 2001a). It is one of the molecular switches that control the fate of cells, as it interacts with many factors including insulin-like growth factor 1 receptor/Src/MAPK/Egr-1 signaling pathway that results in prosurvival (Criswell et al. 2003a; Criswell et al. 2003b). The activity of radiation-induced clusterin can be suppressed by p53 suppressor proteins, which shows that it is linked into the p53 pathway (Criswell et al. 2003a; Criswell et al. 2003b). Nuclear clustetin/X1P8 can bind to Ku70, modify cell proliferation (Yang et al. 2000b; Yang et al. 2000a), and signal the induction of cell death (Yang et al. 2000b; Yang et al. 2000a).

Secretory clusterin protein is implicated in aging, obesity, heart disease, and cancer. Regulatory signaling transduction processes control secretory clusterin expression. Secretory clusterin plays an important role in many of the radiation responses (bystander effects, genomic instability, and adaptive responses) induced by low doses of radiation (Klokov et al. 2004). Radiation exposures and the signal protein, TGF-ß1, increase the production of secretory clusterin. Radiation exposure causes stress activation of signaling pathways that regulate the clusterin cascade pathway and can result in apoptosis and antiproliferation signaling, which can result in killing of prostate cancer cells *in vitro* (Shannan et al. 2007). However TGF-ß1 can override this suppression and allow massive expression of secretory clusterin. Furthermore, adding secretory clusterin to medium suppresses TGF-ß1-induced cell growth. It has even been suggested that the

level and type of clusterin present in a system after exposure to ionizing radiation can be used as a marker of radiation exposure (Klokov et al. 2003).

(Kalka et al. 2000) suggested that secretory clusterin can play an important role in modifying responses to tumor therapy by inducing apoptosis and helping to eliminate skin tumors following phthalocykanine 4 photodynamic therapy. It has been shown to be an excellent candidate for changing radiation resistance in prostate cancer and may be useful in other types of cancer therapy. Research is under way to get clusterin into clinical trials (Shannan et al. 2006). Understanding the mechanisms involved in the action of clusterin in both high and low dose ranges has increased its usefulness in cancer therapy as well as its role in estimating radiation-related cancer risk

F. Changes in Gene Expression

Given that the spectrum of changes induced by low levels of radiation differs from those induced by high radiation doses (Robson et al. 1997; Hande et al. 2003; Tomascik-Cheeseman et al. 2004) the major questions that remain are about the biological impact of these radiation-induced changes. Are the changes induced an indication of an increased cancer risk induced by low doses of radiation, or are they changes that protect the biological system, which would lower the cancer risk in the low dose region?

The rapid advances in the fields of genomics and proteomics both within and outside the Program have provided the tools to understand the biological responses induced by low doses of ionizing radiation and the significance of these changes. Program research determined that many pathways controlling the fate of cells were modified by a range of different chemicals and that low doses of radiation changed the expression of the genes and proteins associated with these chemicals (Azzam et al. 1998; Amundson et al. 2002; Coleman et al. 2003a; Yin et al. 2003; Lyng et al. 2006; Amundson et al. 2008).

As discussed earlier, the observations that radiation can produce DNA damage, mutations, chromosome aberrations, and genomic instability suggests that radiation is harmful at all doses. However, recent research demonstrated that many mechanisms are involved in cancer induction that influence the shape of the dose-response relationships. Extending these recent observations on non-DNA damage related changes into the low dose region and the relationships that exist between changes in gene expression and the intital changes in DNA damage remain critical research areas. The importance of radiation-induced genomic instability and changes in gene expression has not been widely researched. (Snyder & Morgan 2004a, b) determined that changes in gene expression profiles change rapidly after radiation exposure making it hard to relate these early changes to the development of late occurring diseases. Studies were conducted to determine unique changes in gene expression in an attempt to understand the mechanisms involved in the induction of genomic instability, and few clues were found as to the initiation and perpetuation of chromosomal instability (Snyder & Morgan 2004b, a). Expansion of these types of studies may be necessary to link genomic instability to mechanisms of actions for radiation-induced changes in the low dose region and to understand the net effects of low doses of radiation on risk.

Research suggests that even though DNA damage is induced linearly as a function of radiation dose, the processing of this damage, the signaling induced by the damage, and the biological consequences of the damage change as a non-linear function of dose. Low doses of radiation were thought to be involved in modification and repair of DNA damage. Early studies with gene expression failed to demonstrate that modified expression of DNA repair genes was altered by low doses of radiation (Tomascik-Cheeseman et al. 2004). It was also determined early in the Program that DNA-dependent protein kinases do not play an important role during the induction of adaptive responses (Odegaard et al. 1998). Thus, direct induction and repair of DNA damage may not be as important in the total risk from low doses of ionizing radiation as other cellular processes.

Radiation-induced changes in gene expression suggested that many cellular processes were influenced by exposure to low doses of radiation. Modification of genes involved in control of the cell cycle, changes in oxidative metabolism, and modification of signaling pathways were altered by low doses of radiation. These changes are reflected in adaptive responses produced by both low total doses and low radiation dose rates. Such changes have been shown to result in protection and sometimes even in the decrease in biological damage below the level observed in control cells (Elmore et al. 2008).

Several genes have been identified that are induced by low doses of ionizing radiation and suggest a potential mechanism involved in the protective effects. (Okazaki et al. 2007) showed that the genes involved in p53 and p53-related pathways are modified by low doses of radiation and play an important role in the production and modification of apoptosis. Apoptosis has been linked to radioadaptive responses and to the elimination of transformed cells.

Genes involved in the production of Mn(SOD), known to be involved in radiation protection, are up-regulated by low doses of radiation and play an important role in the induction of the adaptive responses (Guo et al. 2003). This provides another area of research that suggests that low doses of radiation can be protective. Up-regulation of Mn(SOD) has been suggested to reduce the level of damage observed in the systems exposed to low doses of ionizing radiation to levels below that observed in the non-exposed controls.

The level of glutathione can also reduce the frequency of chromosome aberrations, modify cell cycle kinetics and protect mouse bone marrow cells following ionizing radiation exposure (Ray & Chatterjee 2007). Glutathione is also up-regulated in the low dose region. Modification of energy metabolism has recently been linked to changes in cellular responses to ionizing radiation. It was demonstrated that treatment of cells with 2-Deoxy-D-glucose, an important marker of modification of energy metabolism, causes cytotoxicity, oxidative stress, and radiosensitization in pancreatic cancer cells (Coleman et al. 2008). PARP-1 and PARP-2 are changed by low doses of ionizing radiation and play a role in radiation-induced resistance and protection (Chalmers et al. 2004).

All of these studies need to be integrated in a meaningful way and used in modeling studies as we continue to evaluate the influence of low doses on cancer risk. These types of studies provide early insight into the mechanisms involved in low dose radiation effects.

Studies with microarray techniques have also provided mechanistic information on how cells communicate with each other following low dose radiation exposure. The activation of communication pathways seems to be one of the major responses to low doses of ionizing radiation. Such studies show that cells, tissues, and organs communicate and respond to radiation in an integrated fashion rather than as individual cells. Suggestions on current pathways involved in communication as well as insight into future research directions are needed to better understand how such communication will influence radiation responses (Chaudhry 2006).

Studies on radiation-related changes in gene expression have also been applied to understanding the cancer process. Such studies have not been a major focus in the Program, so they are not reviewed in detail here. However, several research studies funded by the Program have suggested strong correlations between changes in gene expression and cancer induction.

(Park et al. 2005) conducted studies determining that the susceptibility of cancer cells to B [beta]-lapachone is enhanced by ionizing radiation. The same research team also noted that upregulation of NAD(P)H: quinine oxidoreductase by radiation exposure potentiated the effects of bioreductive [beta]-lapachone on cancer cells (Choi et al. 2007). This suggests potential pathways for cancer treatment and control using combined radiation and chemical treatment. It may be possible for some of these combined treatments to be moved into the clinic and provide potential methods to improve cancer therapy.

Gene expression profiles were also used in association with studies that identified chromosome translocations using SAGE that were important in the development of myeloid leukemia (Lee et al. 2006). Such studies are important in providing direct pathways between molecular studies, cellular changes and the development of disease. With this type of information it will become possible to follow the early changes in gene expression and link them to the cellular outcome and, finally, the late-occurring diseases such as leukemia. Such studies on gene expression suggest that there may be radiation-induced genes that may be exploited in the development of gene therapy (Greco et al. 2005).

Molecular and cellular biology continue to provide powerful tools for understanding the biological responses to radiation, especially in the low dose region. The potential protective effects of such responses, especially in the low dose region, have been reviewed and their impact on risk evaluated (Tapio & Jacob 2007). These studies also play an important role in developing methods for radiation protection and were reviewed in a workshop designed to evaluate their use as biomarkers as well as to more fully understand mechanisms involved in radiation protection (Coleman et al. 2003a; Coleman et al. 2003b). Using these techniques to obtain data and relating the results to biological outcomes, especially late-occurring diseases, is an important area for continued research.

G. ROS Status of the Cell

Research conducted within the Program emphasized the important role of the reactive oxygen status (ROS) of the cells and oxidative stress in the development of radiation-related disease (Azzam et al. 2002; Murley et al. 2002; Azzam et al. 2003a; Gius & Spitz 2006). In the low dose region, it was determined that protective mechanisms were activated that involved changes in the mitochondria, the ROS status of the cells, and the modification of radioprotective chemicals,

including the well-known SH containing radioprotective chemicals. This research provided an excellent link between low doses of radiation and the observed biological responses such as adaptive response, bystander effects, and genomic instability.

Free radicals play an important role in the induction of cancer and other diseases of aging, and radiation produces free radicals and oxidative stress. Research on the impact of radiation-induced free radicals has focused on the alterations in DNA and the induction of chromosome damage and mutations as the result of radiation exposure. A major goal of the Program was to determine the differences between biological alterations produced by free radicals during normal oxygen metabolism and those produced from ionizing radiation, as discussed in Chapter 4. It was suggested that DNA clustered lesions were unique to ionizing radiation and could act as a marker of radiation induced damage. Some research suggested that this may not be the case and that factors such as endogenous oxidative damage produces clustered DNA lesions in unirradiated viral DNA and in human cells (Sutherland et al. 2003c; Sutherland et al. 2003b).

Oxidative stress, ATM deficiency, and normal cellular metabolism all seem to be important for the repair of DNA damage (Barzilai et al. 2002). It was determined that repair of DNA damage was defective when the above conditions are present, illustrating again that genetic background is one of the most important elements of the responses associated with radiation exposure. The ROS status of the cells was also determined to be critical in the induction of both DNA damage and error-prone DNA repair. This was put forward as a model to link genomic instability and the progression of myeloid leukemia (Rassool et al. 2007). In contrast to these studies, it was also determined that radiation can induce a number of proteins important in DNA DSB repair (Leskov et al. 2001a; Leskov et al. 2001b).

Thus, radiation can both induce DNA damage and help repair it. Such observations support data that demonstrate the fact that DNA damage increases as a linear function of radiation dose but the processing and repair of that damage is non-linear and seems to result in total dose-response relationships for many endpoints that are sub-linear.

1. ROS and genomic instability

There are strong relationships between environmentally induced stress, cellular oxidative stress, chronic inflammation, and the induction of cancer. Diseases such as chronic esophageal acid reflux syndrome result in an inflammatory disease of the esophagus, a major risk factor in the production of esophageal cancer. Thus, the role of ROS in maintaining genomic stability has been the subject of Program research.

Large doses of ionizing radiation cause extensive damage at the molecular level, which causes cell killing and then major tissue disruption. A primary outcome of these high doses is alteration of the ROS status of the cells and tissues with marked increases in radiation-induced free radicals. (Limoli et al. 1998) determined early in the Program that changes in the ROS status of cells in culture were important in the production of apoptosis and reproductive failure of cells. These changes were evident in cells with a compromised genomic integrity (Limoli et al. 1998) and resulted in chromosomally unstable cells (Limoli et al. 2003). The dose-response relationship for such changes has a rather steep slope, and in the high dose region seems to increase linearly with radiation dose.

Because of the high frequency of radiation-induced genomic instability (Limoli et al. 2003), early studies suggested that individual genes were not one of the major causes. However, (Slane et al. 2006) demonstrated that mutations play a role in genomic instability. In cells with mutations in succinate dehydrogenase subunit C there was a high frequency of genomic instability. In studies of the molecular parameters associated with genomic instability, (Pichiorri et al. 2008) determined that genes and cells with fragile sites are important in the induction of genomic instability.

In addition to changes in DNA-associated mutations, other factors play an important role in the induction of genomic instability. Interleukin 8 produced a pro-mitogenic and pro-survival role in radiation-induced genomically unstable cells (Laiakis et al. 2008). This change in cell kinetics, which resulted in survival of damaged cells, was previously suggested as a mechanism for the selection of radioresistant cell variants seen in genomically unstable cell lines (Limoli et al. 2001a). Together, these results support the role of cell selection in the development of radiation-induced genomic instability.

In addition to selection of cells in genomically unstable lines, cell senescence is an important player. Studies demonstrated that senescence, cell transformation, and genomic instability were all mediated by platelet/megakaryocyte glycoprotein Ib alpha, indicating the importance of this factor in monitoring and maintaining the stability of the genome (Li et al. 2008a; Li et al. 2008b).

Radiation-induced changes in ROS acts as a trigger to initiate cross talk between the ROS status of the cell and NF-κB. This cross talk results in a number of molecular changes that have biological significance (Bubici et al. 2006). Some of the suggested biological changes produced by the interaction of NF-κB with oxidative stress signaling are the production of genomic instability (Natarajan et al. 2007). It has also been demonstrated that in chromosomally unstable cell lines there is a differential induction and activation of NF-κB complexes (Snyder & Morgan 2005a, b). This again provides additional information regarding the mechanisms involved in radiation-induced genomic instability and, potentially, radiation-induced cancer. As discussed in Chapter 5, additional research is needed to link the induction of genomic instability to the formation of cancer.

The relationships between normal metabolism, mitrochondrial dysfunction, levels of reactive oxygen species, and radiation-induced genomic instability have been carefully reviewed (Kim et al. 2006). At high doses, these relationships have been well established. It is critical to extend and expand the research on the association of genomic instability and the ROS status of the cells into the low and very low dose regions. Such studies will be key in determining if low doses of radiation result in protective mechanisms that stabilize the genome, while high doses result in genomic instability. The important question that remains to be addressed is “do low doses result in responses that increase or decrease the ROS status of the cells?” The answer to this question would provide a potential mechanistic basis for understanding the differences in the biological responses to high and low doses of radiation and an explanation about how radiation can either protect against or enhance the induction of genomic instability.

2. ROS and adaptive responses

The role of cell ROS status on the induction of adaptive responses remains an important area of research, because it could have a major impact on the shape of the dose-response relationship and risk in the low dose region. As research has progressed from single-cell *in vitro* studies to more complex cell and tissue relationships, it has become evident that both tissue architecture and oxidative metabolism are a critical part of the induction of adaptive responses (de Toledo et al. 2006). These protective responses are linked to mitochondrial function.

It has also been demonstrated that mitochondrial DNA repair is important in the induction of cellular resistance to oxidative stress induced by a number of environmental and experimental conditions (Grishko et al. 2005). The role of the mitochondria in the total radiation response also has been found to be very important. Ionizing radiation alters cyclin B1, which is involved in control of cell cycle. This alteration seems to be regulated through NF-κB and the antioxidant enzyme MnSOD, which can modify the oxidative status of the cells and act as a protective mechanism against radiation-induced damage (Ozeki et al. 2004).

Using mouse skin epithelial cells, (Fan et al. 2007) showed that the adaptive response is dependent on the interaction between NF-κB and MnSOD, producing a decrease in the ROS status of the cells. The relationships between MnSOD, NF-κB and the adaptive and protective responses have been advanced through research directed toward developing radioprotective compounds. Radioprotective drugs have been used during cancer radiotherapy to protect normal cells against radiation injury (Murley et al. 2004) determined that the production of MnSOD was one of the major pathways altered by the treatment with drugs such as the free thiol form of anifostine. By using experimental protocols that gave repeated administration with this form of anifostine, radioresistance could be maintained, and the level of MnSOD was elevated and seemed to be important in this continued radioresistance (Murley et al. 2007; Murley et al. 2008).

Other research has demonstrated that by altering the ROS status of the cells it is possible to provide radiation protection against a wide range of different radiation types with a range of different LET such as that which would be encountered during space exploration. In these studies, both antioxidants and Bowman Birk proteins resulted in marked reduction of the free radicals in cells and protected against radiation- induced damage (Kennedy et al. 2006). Such studies suggest that radioresistance observed in MCF-7 breast cancer cells is related to the ROS status of the cells as well as the level of peroxiredoxin II in the cells (Wang et al. 2005). These are experimental variables that may be altered by experimental treatments.

Proteomic and transciptomic analyses have helped determine that mitochondrial dysfunction results in the induction of oxidative stress in cells leading to cell killing through apoptosis (Chin et al. 2008). Additional research demonstrated that apoptosis can be modified by many factors that modify the oxidative stress and ROS levels in the cells and tissues. These studies were conducted in mouse models of Parkinson's disease but the results may be applicable to other forms of diseases that are associated with increases in oxidative stress such as radiation-induced cancer in the high dose range.

(Dong et al. 2007) found that treatment of cells with vitamin E analogues altered oxidative status levels and can induce selective apoptosis in proliferating endothelial cells and stop angiogenesis, which is critical in cancer growth and spread. Such research highlights the role of normal oxidative metabolism and suggests that alterations of this metabolism by any type of stress can be either protective or detrimental in the risk of cancer development. It was also demonstrated that any form of stress may result in stress-induced premature senescence (SIPS) (Suzuki & Boothman 2008), which may play an important role in aging as well as the loss of genomic stability and cancer development. Research in this area could provide mechanist data to link some of these biological observations and help understand how they may be altered by experimental treatment. (Andringa et al. 2006) suggested that altering the metabolism may potentially sensitizing cancer cells to radiation and to the toxicity of 2-Deoxy-D-Glusose).

The observations described here demonstrate the critical role of the redox status of the cell in cancer biology. This subject has been reviewed by (Gius & Spitz 2006), as has the role of stress and how it alters gene expression, senescence, redox status of the cells and the risk for cancer, and links between these factors have been established (Denko & Fornace 2005). All this research makes it clear that the ROS status of the cells is critical during cancer development and that the responses to radiation in altering this status is very dependent on the radiation dose. High doses increase the stress and reactive oxygen levels in the tissues and cells and increase risks, while low doses seem to increase the level of MnSOD, which would protect cells and possibly reduce cancer risks.

G. Cellular Changes

1. Chromosome aberrations

Radiation responses need to be extended and coordinated across different levels of biological organization from the initial changes in gene and protein expression to changes in the ROS status of the cell and morphological changes in cells, and then to linking these changes to disease. The first of these cellular changes to be discussed is chromosome aberrations. Measurement methods and the impact of scoring chromosome aberrations were discussed in in Chapter 4, and itt has been well established that scoring chromosome aberrations is very useful in biodosimetry. Studies on chromosome aberrations also supply critical new information on the mechanisms of action of radiation as a function of radiation type, dose, dose rate, and dose distribution. Most tumors have abnormal chromosomes with either translocations, duplications, losses, or changes in total chromosome number, and studies of these changes have helped evaluate their role in cancer induction. Continued research is needed to understand radiation- induced chromosome aberrations and their role in the development of cancer, particularly leukemia.

2. Telomeres

Another recent discovery about radiation-induced changes in chromosomes is the role of the telomere in radiation-induced biological damage. New staining techniques made it possible to mark the different telomeres on each chromatid and detect radiation-induced changes in the telomeres. With these techniques, it was possible to demonstrate unique differences, which were dependent on the DNA strand associated with them. (Bailey et al. 2001b) performed post-replication processing of the telomere that was dependent on the DNA strand, so that it could be

determined which strand each telomere was associated with. (Zou et al. 2004) determined that replication of each telomere's DNA was asynchronous and was again dependent on the strand of the DNA involved. These processes made it possible to identify each telomere and to follow changes induced in the telomeres by experimental procedures providing a very useful tool for extensive mechanistic research on cellular radiation effects.

Telomeres play a critical role in the proliferative life of cells. As cells and organisms age, the length of the telomere decreases. The protein telomerase is involved in the process of maintaining telomere length, and in early research, it seemed to be essential. Telomere length and maintenance also changes in transformed and cancer cells. Thus, it is important to be able to understand how telomere length is maintained to control the fate of the cells. (Nugent et al. 1998) demonstrated that telomere maintenance is also closely linked to end repair of double strand-breaks in DNA. Further work demonstrated that DNA DSB proteins were required to cap the ends of mammalian chromosomes during the formation of telomeres (Bailey et al. 1999). The activity of DNA-PK kinase was found to be essential in protection of the mammalian telomeres (Bailey et al. 2004b; Bailey et al. 2004d).

Even though telomerase is essential in the maintenance of telomere length (Lee et al. 1999) determined that many of the DNA repair enzymes could act through independent DNA repair pathways to maintain telomere length in the absence of telomerase. This research was extended to *Saccharomyces cerevisiae* where the role of DNA repair on the telomere length was supported (Lee et al. 2002). (Bailey et al. 2004b; Bailey et al. 2004d) also demonstrated that frequent recombination in telomeric DNA could extend the life and maintain telomere length in cells that were telomerase negative. Such observations provide strong links between DNA repair and the maintenance of the telomere (Bailey et al. 2001b; Bailey & Goodwin 2004). When there was a defect in the telomeres that was present at the same time as DNA DSBs induced by ionizing radiation or other environmental insults, there was interaction between the DNA DSBs and the telomere (Bailey et al. 2004c). This interaction has been reviewed, and it seems to be a very general biological interaction and plays an important role in maintaining the stability of the genome (Bailey & Cornforth 2007).

It has been postulated that radiation-induced genomic instability is important in the generation of cancer. Since genomic instability occurs at a very high frequency, it was critical to look for targets larger than traditional radiation-induced gene mutations for the induction of genomic instability. As research on telomeres has advanced, it has been linked to the induction of genomic instability induced both in cell systems and in animal model systems. The telomere provides a larger target and the higher frequency needed to explain radiation-induced genomic instability. Studies with a mouse model (K-ras p53) on the induction of lung cancer indicated that telomere dysfunction promotes genomic instability as well as increasing the metastatic potential for the cancers (Perera et al. 2008). Reviews of the literature on the inter-relationships between genomic instability and telomere dysfunction suggest that telomere dysfunction is one of the major driving forces in radiation-induced genomic instability (Bailey et al. 2007).

It is important to link radiation-induced changes in telomeres to radiation exposures. (Durante et al. 2006) demonstrated that, following radiation exposure with heavy ions that result in very high deposition of energy per unit of distance traveled in the tissue, there are chromosomes that lack

telomeres. This would suggest the breakage and loss of genetic material and alteration of the telomeres that could be important in loss of genomic stability.

(Zhang et al. 2005) demonstrated that by suppressing DNA-PK activity using RNAi that both telomere dysfunction and mutation frequency were altered. This effect was dependent on the type of radiation used in the studies, with HZE particles found in space being more effective that gamma rays in producing these effects.

Radiation exposure produces DNA damage, changes in telomere function, and genomic instability. An important review of these effects on telomeres, chromosome instability, and cancer suggested strong causal links between these observed cellular and subcellular changes and the induction of cancer (Bailey & Murnane 2006). Such studies provide a potential mechanism for the induction of cancer through a radiation-induced telomere dysfunction and genomic instability. Because the induction of genomic instability by higher radiation doses increases linearly with radiation dose, such a model supports the LNT hypothesis at these doses.

3. Cell Cycle

Early in the field of radiation biology, scientists recognized that radiation could cause blockage of the cell cycle at specific stages of the cell cycle. It was postulated that the blockage of the cell cycle in the G_2 stage of the cell cycle allowed additional time for DNA repair before the cell progressed through mitosis and "set" the damage. This was thought to be a protective mechanism that decreased the damage and risk from radiation. Ku and CHK1-dependent radiation-dependent G_2 blockage were evaluated to define the pathway and mechanism involved in initiating the cell cycle delay (Wang et al. 2002).

Research focused on other radiation-induced biological changes determined that the cell cycle plays an important role in several of the new observed low dose biological phenomena such as adaptive response and low dose radiation hypersensitivity. (Ahmed & Li 2008) determined that Cyclin D1 was a critical actor involved in altering cell cycle and the induction of the adaptive response. This research was in contrast to much earlier research that failed to show that the cell cycle was an important variable in adaptive response. Alterations in the cell cycle were very critical during the induction of low dose radiation hypersensitivity and that the population of G_2 cells were a primary factor that resulted in cell killing in the very low dose region of the dose-response relationships.

Research on the role of reactive oxygen on low dose responses determined that normal regulation of cell cycle progression was altered by flavin-containing oxidases that may be influenced by low doses of radiation (Venkatachalam et al. 2005). Studies using compounds such as isobuosilactone A, which alters ROS and induces apoptosis, also suggest that radiation may alter cell cycle progression. Such compounds modify the kinase 1 signaling pathway that is critical in the induction of human breast cancer (Kuo et al. 2009). The direct association between these observations and the low-dose radiation responses has not been established and is another area of needed research.

New techniques have been developed to follow cell proliferation and clonal expansion using integrated one- and two-photon imaging. Such studies suggest that clonal expansion may be a major factor in determining mutation load in cells and tissues and may be important in the development of radiation-related cancer (Wiktor-Brown et al. 2008).

(Wilson et al. 2004) published a useful review on the role of radiation and cell cycle progressions. As more mechanistic information becomes available on the role of radiation in cell cycle progression, clonal expansion and cell cycle changes, the influence of cell proliferation kinetics on cancer risk may be forthcoming.

4. Hyper-radiation sensitivity and radiation resistance

Past research at higher radiation doses suggested that cell killing was a simple decreasing function with dose. However, it was determined that cell killing increased rapidly as a function of low doses exposures (hyper-radiation sensitivity, HRS). As the dose increased the cells became radiation resistant and the cell-killing slope decreased as dose increased IRR. Subsequently, numerous studies were conducted to help define the mechanism of action involved in these unique low-dose radiation responses. Early studies focused on the influence of external conditions on the induction of the response, such as oxygen tension (Marples et al. 1994) and dose fractionation (Short et al. 2001). Other studies focused on the potential genes and proteins involved in the responses(Marples et al. 1997; Marples & Joiner 2000; Marples et al. 2002; Chalmers et al. 2004). As is often the case, many mechanistic studies related to HRS and IRR induction were negative; that is, the endpoint studied did not influence the shape of the dose-response relationship in the low dose region. Further studies determined that ATM Se1981 had no influence on the shape of the cell killing curve in the low dose region (Krueger et al. 2007b; Krueger et al. 2007a), and that the recognization of DNA DSBs was not related to the induction of HRS (Wykes et al. 2006).

(Krause et al. 2005a; Krause et al. 2005b) determined that low dose hypersensitivity did not influence the cure of cancers in experimental animals and did not translate into an improvement outcome in ultrafractionationed radiotherapy *in vivo*. On the positive side it was suggested that low dose hypersensitivity is associated with p53-dependent apoptosis. In addition, extensive studies conducted determined that the stage of the cell cycle at the radiation exposure was very important in low dose hypersensitivity. Cells in the G_2 stage of the cell cycle were most sensitive to low dose hypersensitivity followed by radiation-induced resistance (Marples et al. 2003; Short et al. 2003; Marples et al. 2004).

The literature on low dose hypersensitivity and radiation-induced resistance has been carefully reviewed at different times (Joiner et al. 2001; Joiner 2004; Marples & Collis 2008). These phenomena are very important observations relative to the shape of the dose-response relationships in the low dose region. If low doses of radiation increase cell killing, this treatment could be eliminating cells from the population that may be at higher risk for the induction of cell transformation. Conversely, low dose hypersensitivity and radiation-induced radiation resistance to cell killing could increase cell proliferation in the low dose region and protect cells that are sensitive to radiation-induced cell transformation in the medium dose range. It could be postulated that this combination could result in an increase in cancer risk in the low dose region.

Such increases above that predicted by the LNT have not been detected in any animal or human studies and do not seem to be a viable postulate.

5. Apoptosis

The process of apoptosis, or programmed cell death, has been recognized for a long time and plays a critical role during embryonic development. As cells differentiate and form organs, many of them are programmed to die. For example, in the formation of the hands, the cells between the fingers die on a pre-programmed schedule, allowing the fingers to separate. During the early days of radiation biology, it was not widely recognized that radiation produced apoptosis. Cells were thought to be killed by radiation through either the processes of mitotic death or necrosis. However, it was recognized before the initiation of the Program that apoptosis played an important role in cell killing of lymphoblastoid type cells following exposure to radiation (Schwartz et al. 1995).

This radiation-induced apoptosis had been called interphase death, and lymphocytes seemed to be the major cell type that suffered from this type of death. As the research continued, it became obvious that death of the cells during the G_0 stage was indeed apoptosis and that radiation was a major factor. Studies were conducted to determine the dose-response relationships for the induction of apoptosis. These suggested that for non-genotoxic insults a threshold existed for both radiation-induced cytotoxicity and apoptosis, below which little apoptosis could be observed in human lymphocytes. (Schulte-Hermann et al. 2000) suggested that "Applications of non-genotoxic carcinogens at doses too low to interfere with life-death decisions of cells or for time periods too short to cause irreversible transitions in cell populations may therefore be considered below the biological threshold for a carcinogenic effect."

Because radiation is classified as a genotoxic carcinogen, it was suggested that such responses do not apply to ionizing radiation. Studies using radiation demonstrated that many systems are very sensitive to radiation-induced apoptosis, and no threshold could be detected below which there was no response. It was also determined that, at very low doses, the induction of apoptosis may be protective for the induction of cell transformation and other endpoints of interest for cancer risk (Mendonca et al. 1999). Dose-response studies were conducted in lymphocytes for the induction of apoptosis, and it was determined that the RBE for 280 keV neutrons relative to X-rays was approximately one (Ryan et al. 2006). This suggests that neutrons were similar in effectiveness in the production of damage or the triggering of the signaling pathways associated with apoptosis.

Because the process of apoptosis was well recognized in the embryo, it was a logical extension to evaluate radiation's role in the induction of apoptosis during embryogenesis. Such a role could be important in normal development and suggests a potential role of radiation-induced apoptosis on the induction of birth defects that has been well documented at set stages of development (Hall 2000b). This sensitive stage of embryogenesis is when the limbs and organs are undergoing critical changes during development. Radiation induces apoptosis during embryogenesis, and this could provide a basic mechanism for induction of radiation-related developmental abnormalities (Bladen et al. 2007b).

As is the case for most biological responses, the genetic background of the individuals or cells is critical to determine the magnitude of the radiation response. Early studies of the induction of apoptosis in lymphocytes demonstrated that the p53 status of the cells played a major role (Schafer et al. 2002). These observations were extended to whole animals, and it was determined that the p53 status of mice played a major role on radiation-induced changes in gene expression. These radiation-related changes in gene expression seemed to alter the frequency of apoptosis and could play an important role in repair of DNA damage and removal of cells with damaged DNA (di Masi et al. 2006). Modification of the genetic background of mice to produce a haploinsufficiency for ATM and RAD9, a DNA repair gene, resulted in marked changes in radiation-induced repair of DNA damage. These changes in repair modified other biological processes that were reflected in changes in the frequency of apoptosis and cell transformation (Smilenov et al. 2005).

As additional studies were conducted on radiation-induced transcriptional activity of genes in the thymus and spleen of humans, it was noted that there was a high sensitivity for radiation-induction of genes involved in the two major apoptotic pathways (Alvarez et al. 2006). This provides links between radiation-related gene induction and the production of apoptosiss an important role in cancer risk. It has also been demonstrated that experimental modification of p53 such as with acetlyation at lysine 317 can negatively regulate apoptosis and modify cell survival (Chao et al. 2006). Even though the p53 status of the cells and animals seemed to play a major role in the induction of apoptosis, (Takahashi et al. 2005) determined that for high-LET radiation the p53 status did not influence the outcome. Exposure to high-LET radiation may create such a marked biological alteration that the signaling and damage induced act independently from the p53 status. For low-LET radiation exposure there is no question that the p53 status of the cells and organisms is extremely important to trigger the induction of apoptosis following DNA damage. Thus, it is well established that p53 is important in the signaling pathways that are activated by DNA damage and result in apoptosis.

As previously discussed, another important component of the radiation-induced signaling pathway is NF-κB. Radiation-induced NF-κB is directly associated with apoptosis (Meng et al. 2003), and it has been suggested that blocking the NF-κB pathway can alter the frequency of apoptotic cells. Using different types of DNA-damaging agents, it was possible to further link NF-κB to radiation-induced DNA damage and apoptosis. The type of DNA damage influenced the frequency of apoptosis (Strozyk et al. 2006).

A major goal of the Program was to determine if there was a difference between DNA damage from endogenous factors and that induced by radiation. (Li et al. 2008a) tested the hypothesis that there was a difference between the frequency of apoptosis induced by radiation-related DNA damage and that induced by DNA damage from endogenous sources. They demonstrated that complex DNA damage was more effective in production of apoptosis than simple DNA double strand breaks. Such observations suggest that radiation-induced complex DNA lesions may play a critical role during radiation-induced apoptosis.

Following radiation-induced DNA damage, many repair genes and processes determine the signaling initiated and fate of the cells with DNA-damage. Many of the signaling pathways that result in apoptosis have been identified, characterized, and modified by experimental protocols.

It was determined that activation of the TNF-related apoptosis-inducing ligand (TRAIL) gene was important in the induction of apoptosis (Kagawa et al. 2001). (Aravindan et al. 2008) measured the length of time that the signaling processes and molecules involved in apoptosis exist following radiation exposure. The protein-kinase signaling pathway resulting in radiation-induced apoptosis can be modified by both H-ras and Ki-ras. This modification can result in an increase or decrease in the frequency of apoptosis depending on the genes involved and the pathway that is induced (Choi et al. 2004).

It was also possible to determine how the signaling pathways induced by radiation change as a function of time after exposure (Aravindan et al. 2008) as well as a function of the type of radiation exposure (Ryan et al. 2006). Many molecules and DNA repair proteins also play important roles in apoptosis. For example, staurosporine modulates radiation-induced apoptosis, and ceramide is also required for radiation-induced apoptosis (Guo et al. 2006; Deng et al. 2008). It is important to recognize that some of these studies were conducted *in vitro* and others in non-mammalian systems. Additional research is required to determine if such compounds will be important in radiation-induced apoptosis in humans.

To better relate apoptosis to cancer, studies have measured the induction of genomic instability and related it to the induction of apoptosis. Radiation-induced apoptosis profiles in cells that were genomically unstable were different from the induction of apoptosis in normal cells (Nagar et al. 2005). Other studies relating genomic instability to apoptosis determined that both could be induced by exposure to either carbon ions or X-rays (Ryan et al. 2006).

Genomic instability is a step in the carcinogenesis process and may be directly related to the induction of radiation-induced cancer. It has been demonstrated that the frequency of radiation-induced genomic instability increases linearly with high doses. The role of apoptosis in this process is complicated because the removal and loss of damaged cells by apoptosis may reduce cancer risk.

Apoptosis has been demonstrated to be a frequent event following exposure to low doses of ionizing radiation, and seems to be an important part of the adaptive responses observed following these exposures. Experimental conditions that decreased the frequency of apoptotic cells increased the frequency of APRT mutations in mice repeatedly exposed to ionizing radiation (Liang et al. 2007). Radiation resistance can also be increased by factors that modify cell cycle and reduce apoptosis (Park et al. 2000). Such studies suggest the potential for apoptosis to be protective against late-occurring diseases such as cancer. This observation has been related to the induction of protective or adaptive responses in the low dose region of the dose-response curve.

One of the most important observations of apoptosis in radiation biology is the suggestion that low doses of radiation can trigger biochemical and signaling pathways in bystander cells that result in selective apoptosis of cells that are transformed and may be in the process of changing from normal to cancerous cells (Bauer 2007a; Portess et al. 2007). If low doses of radiation can selectively cause transformed cells to undergo programmed cell death, then it has been postulated that the cancer risk can be directly reduced (Portess et al. 2007). This would help explain experimental results in the study of cell transformation where low doses of ionizing

radiation decrease the frequency of transformed cells below the levels seen in controls (Redpath 2006b, a). Similar results on the induction of mutations could be explained by this apoptosis-related process (Sykes et al. 2006b; Sykes et al. 2006a; Zeng et al. 2006). As will be evaluated in greater detail in the chapter on modeling, such biology could result in non-linear low dose responses with low doses producing less cancer risk than is present in a non-exposed population (Scott 2007).

Research has demonstrated that many environmental and other factors influence and modify the induction of apoptosis in the low dose region. Survey studies of gene expression in several cell lines demonstrated that the sensitivity to cell killing could be related to changes in gene expression (Amundson et al. 2008). It has also been shown that ubiquitin can up-regulate apoptosis proteins in cancer cells as a protective mechanism against cancer progression (Zhang et al. 2004). The p53 and p53-related genes are associated with protection from apoptosis during the initiation of adaptive responses (Okazaki et al. 2007). The DNA repair gene gadd45a has sensitized epithelial cancer cells to cell killing, which can change the long-term potential for survival of the patients (Lu et al. 2008). It has even been suggested that changes in the diet can modify apoptosis and result in a "suicide solution for the delay of cancer growth" (Khan et al. 2007). All these studies are important in relating cellular changes to risk. They are critical in using systems biology to relate early cellular changes such as apoptosis and cell killing to radiation risk for the induction of cancer.

As is the case for many biological endpoints the ROS status of the cell is a critical variable in the induction and prevention of apoptosis. Very high doses of radiation-induced apoptosis can be modulated by treatment with compounds that inhibit energy metabolism (Hunter et al. 2007). This suggests a direct link between energy metabolism, the ROS status of the cells, and the induction of apoptosis in the high dose region, but provides no information on the responses to low doses. Treating cells with antioxidants decreased the frequency of micronuclei, a potentially detrimental effect of radiation, but it did not affect the induction of apoptosis or the viability of the cells, indicating potentially protective effects (Konopacka & Rzeszowska-Wolny 2006). Research has also demonstrated that treatment of cells with Hsp25 directly inhibited the production of radiation-induced apoptosis by reducing programmed cell death-mediated ROS production.

Changing the ROS status of the cells can be a protective mechanism. Even treatment with vitamin E is thought to inhibit angiogenesis, an essential part of tumor development, by selective induction of apoptosis in proliferating endothelial cells (Dong et al. 2007). Such studies show the importance of apoptosis during cancer development and the role that ROS status and radiation-induced changes in ROS status may have on cancer risk.

The role of apoptosis in radiation oncology has been reviewed (Meyn et al. 2009), as have important factors that control apoptosis such as NF-kB (Dutta et al. 2006) and other pathways that determine whether a cell lives or is killed by apoptosis (Bartek & Lukas 2006). Such information provides for an understanding of the mechanisms involved in radiation-induced cancer in both the high and low dose regions. These reviews and the important scientific publications in this area will be critical as the science of low dose radiation biology moves

forward and attempts are made to use new mechanistic information in evaluation of radiation protection and risk.

IV. Influence of Genetic Background on Cancer Risk

Cancer has long been known to have a genetic component, as many families are cancer prone. The role of genetic background on radiation-induced cancer was carefully reviewed by NCRP (NCRP 2010). This report demonstrates that genetic differences in many molecular, cellular, and experimental animal systems support the role of genetic background on biological responses to ionizing radiation (Williams et al. 2008b; Williams et al. 2008a). Genetic background also impacts the induction of genomic instability (Pichiorri et al. 2008).

Mutations in many genes that contribute to cancer result in the production of chromosomal instability in cell lines (Grigorova et al. 2004). Mutations in many of these genes such as BRACA1, BRACA2, CHK2, and BUB1 are thought to play an important role in the induction of breast, ovarian, and other forms of cancer (King et al. 2003). The relationship between spontaneous cancer and mutations in these genes and radiation-related cancer has been a major area of extended research.

The relationship between genetic diseases and radiation-induced cancer is well established for a limited number of diseases such as Nijmegen breakage syndrome and Ataxia telangiectasia (Little et al. 2002a; Little et al. 2002b). To aid in the study of the relationship between genetic background and radiation-induced cancer, mouse models of Nijmegen breakage syndrome have been developed (Williams et al. 2002). Such studies provide a tight link between genetic background and radiation-induced cancer.

Radiation sensitivity has also been established in primary fibroblasts isolated from families that have hereditary retinoblastoms as well as in many apparently normal controls (Chuang et al. 2006). The differences in these changes are greater when the radiation exposure is delivered at a low dose rate (Wilson et al. 2008). The dose-rate-dependent nature of this response has been used to suggest that repair of DNA damage may be one of the major pathways involved in this geneticially related radiation-induced damage.

Genomic instability has been carefully related to a number of DNA repair deficient mutants in Chinese hamster ovary (CHO) cells (Somodi et al. 2005). Cells defective in homologous recombination DNA repair have been shown to not be sensitive to the induction of sister chromatid exchanges in bystander cells following exposure to low doses of alpha particles (Nagasawa et al. 2008). However, Bystander effects were shown to be dependent on deficiencies in DNA DSB repair (Zhang et al. 2008) suggesting that DNA repair processes are essential in the initiation of bystander responses in chromosomes. Instability in DNA copy number has also been induced by ionizing radiation (Kimmel et al. 2008).

All these studies suggest that genetic background, genomic stability, and radiation-induced DNA damage are closely related. Because ATM is one of the major signaling proteins that responds to DNA damage, the response of genetic background that influenced this protein was evaluated, and ATM heterozygosity did not influence radiation susceptibility to exposure to ionizing

radiation (Mao et al. 2008). This was true both in wild-type background as well as in animals that were heterozygous with respect to their p53 background. These studies become important in establishing the relationships between the effects of genetic background and the role of DNA damage on the activation of communication pathways as an important response to radiation exposure.

This discussion demonstrates that genetic background is critical to radiation response and that there is a range of radiation sensitivity in all cell lines evaluated. Surveys of cell killing and changes in gene expression have been conducted on large populations of different cell types by testing more than 60 cell lines used by the NCI in anti-cancer drug screens (Amundson et al. 2008). Such research suggests that many different genetic factors can influence the induction of cell killing and gene expression, and through these pathways, influence many of the radiation-induced biological responses. The role of radiation on cancer risk is thus very dependent on the genetic background of the individuals and populations being exposed. Genetic background must be carefully considered in any study of the biological effects of ionizing radiation.

Since 2007, the Program has funded some studies of the role of epigenetic effects on the transgenerational responses to ionizing radiation. Epigenetic changes do not alter the DNA but are involved in gene expression, changes in the levels of methylation of DNA, and alterations in protein structure associated with the chromosomes and genes. Such alterations have been shown to alter coat color, metabolism, and cancer risk in mice. Early epigenetic changes in radiation of mice during development are currently being funded. These studies are for the most part in the early stages and represent an important area of current and future research. It has been suggested that epigenetic changes may be related to non-targeted effects of radiation and result in biological changes in cells that do not have energy deposited in them (Kovalchuk & Baulch 2008). If radiation can modify epigenetic changes as well as produce direct changes in DNA, this could have a major impact on cancer risk, especially in the low dose region. These studies must carefully consider dose-response relationships for the induction of epigenetic changes and the implications of such changes on radiation risk.

Major Points: Mechanisms of Action

Extensive research has been conducted on the mechanisms involved in the radiation-induced responses in the low dose region. As the result of this research, the data are now available to explore the magnitude of the risks from radiation-induced cancer in the low dose region. From this research, many interacting processes have been identified that are triggered by low doses of radiation. It will require a systems damage approach to integrate this information into a useful framework to be applied to risk. Important observations that help understand the mechanisms of action in the low dose region are summarized here.

- Biological systems can detect and respond to very low doses of radiation.
- Direct damage to DNA is an important part of the radiation response and increases as a linear function of radiation dose.
- The processing of the damage and the signaling that results from it results in many non-linear processes.

- The signaling pathways induced by DNA damage are important and involve modification of pathways which involve ATM and p53.
- There are multiple genes, chemicals and metabolic pathways induced by low doses of ionizing radiation that have marked influence the biological outcome of the exposure.
- Many of these chemicals and metabolic pathways are protective against radiation-induced damage.
- Low doses of radiation modify the ROS free radical status of the cells. Such modifications are suggestive of radiation protective effects seen in adaptive and protective responses. Higher doses increase the ROS status of the cells to produce responses that are known to damage cells and increase cancer risks.
- In the low dose region, direct radiation effects and the signaling pathways modify cellular responses including cell transformation, mutations, chromosome aberrations, telomere function, and cell cycle delay which seem to be protective. High doses change all these same endpoints in a way that would be predicted to be harmful to the organism.
- Radiation can induce hypersensitivity in the low dose region. As the dose increases there is an induced radiation resistance. Hypersensitivity may be protective by eliminating damaged cells, while induced resistance could be detrimental by protecting damaged cells and allowing them to remain in the population.
- There is evidence that low doses of radiation produce selective apoptosis in cells that are transformed. This provides a major mechanism of action in the low dose region and may help explain many of the adaptive responses observed. Extensive research on the role of apoptosis in radiation risk demonstrates a potential protective role.
- Research has determined that genetic background plays a critical role in all the responses observed in the low dose region.
- Research has been initiated on the role of radiation-induced epigenetic changes.
- There is a need for a more complete view of the relationships that exist between low dose radiation exposure and the cancer process. Without a complete systems approach it will not be possible to apply the current research to radiation protection.

Chapter 7

Modeling

Models are essential in the process of transfering basic data to the needs of regulators. These models are developed at many levels of physical and biological organization. It is essential to understand dose and energy deposition to define the x-axis as well as the biological response that defines the y-axis. The most important data that influence regulatory bodies in standard setting are those associated with human studies. These models are evaluated by regulatory bodies and used widely in standard setting. The role of dosimetric, molecular, cellular and mechanistic data on standard setting still has not been well defined. Models have been developed as part of the Program and provide useful direction and information. Thus, basic mechanistic data can be considered during the standard setting process. Such data and models also can be important in communication of radiation risks. Complex processes can be expressed as models that are easy to understand and communciate.

I. Traditional Cancer Models

To relate biological data to radiation risk it is essential to develop models that describe the data. Radiation risk models in the past have been related to both the cancer process (Moolgavkar & Knudson 1981) and have made primary use of the A-bomb data using the dose-response relationships (Hoel 1987a, b). It was recognized early that cancer is a multistage process (Armitage & Doll 1957) and that to fit the radiation-induced cancer data required a number of variables. However, in any model fitting it is common practice to limit the number of variables to as low a number as possible. Early models would limit the variables to two and get an adequate fit to the experimental data (Moolgavkar 1983).

The development of the generalized formulation of dual radiation action played a role in the evolution of this type of thinking (Kellerer & Rossi 1978). In all model development it was recognized early on that it was essential to relate the variables in the models to real biological changes. It was assumed that these changes were mutations, and extensive research was conducted to determine the mutations essential for cells to transform. It was suggested that it requires at least two mutations to transform a normal cell, and two mutation models were developed (Moolgavkar et al. 1990).

Early in the DOE Program, projects were funded to continue modeling using the responses observed in the low dose regions. Such models were called biologically based models and still are the best approach to using experimental data for model development. The use of the two-mutation model resulted in a good description of the induction of lung cancer in rodents (Leenhouts & Chadwick 1994), and this modeling approach was to describe the radiation-induced lung cancer in rats following radon exposure. These studies suggested that radiation exposure was acting not only on the initiation stage of cancer, where mutations in single cells were thought to be important, but that radiation exposure to the lungs was also essential for the development of cancer, as it acts on the promotion or late stages of cancer development. Such

studies suggested that mutations were not the only changes that were essential in radiation induced lung cancer. These studies were essential in the development of biologically based models (Luebeck et al. 1999). However, the amount of basic biological data available in the Program did not lend itself to modeling efforts, and funding for these efforts was put on hold for about 5 years. As additional data was developed, funding was again made available to model responses from the molecular to the cellular levels and to develop methods linking these models to risk estimates (Brooks 2000b, a). Extensive research efforts continue to be conducted in this area.

These early models concentrated on the biological response observed and related biological average dose to the individual or the organ where the cancer developed. As described in Chapter 6, extensive research on the variables related to radiation exposure was conducted to determine the proper physical properties that needed to be characterized to relate to the biological process. The other concern was the amount and distribution of the energy in very small targets and how this energy distribution would influence biological responses. Research in this area determined that the cell might be the primary target for biological responses; therefore, it would be important to determine the energy and energy distribution in individual cells. This led to the development of the hit-sized effectiveness functions that detailed the amount of energy deposited in individual cells and the distribution of that energy as a function of radiation type and exposure variables (Sondhaus et al. 1996). Such functions were used to understand the relationships that existed between the dose-response relationships and the absorbed dose to individual cells (Bond et al. 1995a).

The extension of the hit size effectiveness factor and other measures of energy deposition to estimate risk were reviewed as a potential exposure metric for ionizing radiation (Bond et al. 1995a; Bond et al. 1995b; Brooks 2005). Such research was supported by developing Monte Carlo track structure codes for low-energy protons to understand the energy and the energy distribution deposited in each cell (Uehara et al. 2001). Additional modeling of the interaction cross sections for intermediate and low energy ions determined the distribution of energy in individual cells from radiation exposure (Toburen et al. 2002).

The development of microbeams helped define the track structure of low-energy electrons (Wilson et al. 2004). These studies were especially important as the low-LET microbeams were developed, and descriptions of the energy and microdosimetric distribution derived for 25-KeV beams were essential (Mainardi et al. 2004). These microbeams made it possible to exposure single cells to known amounts of total energy. A complete description and knowledge of the distribution of that energy within the cells and in neighboring cells was critical (Wilson et al. 2001). This made it possible to develop a useful database on microdosimetry for low-dose, low-LET exposure to ionizing radiation (Wilson et al. 2000). One approach to developing this information was to use Monte Carlo simulations of single cells exposed to known amounts of energy deposited in individual cells from a 25-KeV microbeam (Miller et al. 2001). The information developed from these types of studies made it possible to introduce microdosimetry into the estimation of risk in the low dose region (Scott & Schollnberger 2000). This will be discussed later in the section on models for dose-responses in the low dose region.

II. LNT Models

In the higher dose ranges (>100 mGy), the models provided very good fit to the epidemiology data from the A-bomb (Pierce 2003), which is still the prime source of information used in the development of radiation protection standards. However, as the doses decreased to lower levels the statistical power of the human studies was not adequate and it was very difficult to apply the information to human data to estimate risk. Models were needed that were based on biological and biophysical data to predict risk in the low dose range. In the low dose region, as the dose continues to decrease, the amount of energy in each of the "hit" cells becomes constant, and only the number of cells "hit" will decrease. This thinking is based on target theory where radiation was treated like a gun that shot out energy at the target and deposited it in individual cells (Lea 1955). This was further developed into the "hit" theory. Both theories have been of great historical importance in the field of radiation biology (Zimmer 1961).

If it is assumed that only the "hit" cells are responsible for cancer, and if DNA and mutations represent the prime target for the production of cancer, this results in a model that predicts there will be a linear dose-response relationship in the low dose region, the LNT hypothesis. The history of the development of the LNT hypothesis has been carefully reviewed (Kathren 1996). These assumptions have formed the basis for the models that continue to be used by EPA to estimate risk in the low dose region (Brenner & Sachs 2006) and to set radiation standards (Puskin 2009). The implication from the LNT is that every ionization has the same potential to cause cancer regardless of the radiation dose. The dose-response relationship in the low dose region for the primary source of human data, the A-bomb survivors, supports the use of the LNT and also suggests that there is mechanistic information that supports this hypotheis (Pierce 2003). The scientific basis of the LNT has been reviewed by (Upton 1999; Chadwick & Leenhouts 2005), and review groups (NCRP 2001, NRC 2005). In addition, (Brenner et al. 2003) published a paper that extensively reviewed "What we really know" and supported these assumptions using a mixture of mechanistic information and epidemiological studies.

There is little question that the LNT should be used for setting radiation standards and provides a useful tool to regulating and limiting radiation exposure. However, when the LNT is applied in the low dose region and combined with collective dose measements, it predicts outcomes that may not be scientifically based (Kocher et al. 2008) and results in the public perception that radiation in the low dose region represents a large risk. Extensive data has been developed suggesting that for many biological systems and endpoints there are non-linear dose-response relationships, and there is a large body of scientific data that even suggest that in the low dose region that radiation can protect against genetic damage, cancer, and other diseases. These data suggest that the risk in the low dose region may be less than predicted by the LNT.

How should these data be considered in setting of radiation protection standards? Perhaps LNT is the best we can do today with the information that we have on relating human cancer to radiation exposure in the low dose region (Preston 2003). Continued discussion and debate suggest that even if LNT is useful for setting standards it may not be scientifically accurate for predicting cancer frequency and needs to be constantly re-evaluated.

III. Non-LNT Models

The Program's funding of research in the low dose region produced a large amount of data that has been modeled using non-linear models. As stated above, the primary basis for the LNT was that it was fit by target theory. As additional data has become available on radiation-induced changes in the low dose region, it is obvious that target theory no longer fits the data, especially for non-targeted and adaptive responses. Reviews of these data suggest that target theory modelers can no longer describe the responses in the low dose range (Schwartz 2004).

Many radiation-induced tumor types in humans such as leukemia and bone cancer are best fit to linear-quadratic models, and at low doses many responses seem to have a threshold below which biological changes cannot be detected (Brooks 2006). Some studies on chronic exposure to internally deposited radioactive materials also suggest that lung cancer may have thresholds (Brooks et al. 2009). Threshold models were developed and adequately described the human cancer response to ionizing radiation (Hoel & Li 1998). It has also been demonstrated that the linear-quadratic model is the same as the two-lesion models that were proposed early and that such models also fit much of the radiation-related tumor data (Armitage & Doll 1957).

An abundance of models and other data that suggest non-linear or linear-quadratic responses exists, but because it is difficult to use such models in setting standards, their use has been minimized in considering radiation-induced cancer. The dataset that drives the LNT is related to the observation that when all the solid tumors observed in the A-bomb survivors are grouped, the response is consistant with the LNT hypothesis. However, it has been suggested that there are technical and analytical problems associated with evaluating the A-bomb data that force the function to appear to be linear in the low dose region (Scott 2005a; Scott 2008). Serious debate remains as to whether these really influence the shape of the dose-response in the low dose region.

It has also been assumed by the radiation research community that because mutations, chromosome aberrations, and cancer are all stochastic effects and that stochastic effects increase linearly with dose, all these effects will be related on a one-to-one basis and increase linearly as a function of dose. Research has suggested a mechanistic basis for non-linear induction of stochastic effects (Scott 2005b), and by using Bayesian inference the risk in the low dose range will be non-linear (Schöllnberger et al. 2001).

Extensive research using cell and molecular systems suggest that low doses of ionizing radiation can decrease the responsiveness of cells to subsequent high doses (the adaptive response discussed previously). It has also been demonstrated that low doses of radiation can decrease the normal background frequency of adverse effects. An essential step in carcinogenesis is the transformation of normal cells to cancer cells. The cell transformation system developed by Dr. Les Redpath is a model system that measures the final steps in this process. The data generated from this system has been modeled, and it suggests that low doses of radiation reduce the cell transformation frequency below that observed in the controls. These data would support the use of hormesis as the model system for estimating risk (Redpath & Elmore 2007).

Modeling these effects suggests that there is a decrease in risk in both cell transformation and cancer (Redpath 2005; Redpath 2007; Redpath & Elmore 2007). Research on chromosome inversions has also been modeled and suggests a decrease in risk and a protective effect against

this biological endpoint (Hooker et al. 2004b; Sykes et al. 2006a). Modeling these results also suggested the potential for thresholds for all stochastic effects (Scott 2005b, a). In the low dose region, many factors influence the biological responses and suggest that risk may not be linearly related to radiation dose (Feinendegen et al. 2011).

The extension of these types of results *in vitro* suggest that low doses of radiation may be protective and produce hormesis or beneficial effects. Animal studies have been conducted and were summarized in a review article that demonstrated a change in the latency of several different tumor types produced by low doses or radiation (Mitchel 2006). In this review the author also suggested the potential for low doses to result in a decrease in radiation risk. Other studies suggest a beneficial effect of low dose-rate radiation exposure. (Chen et al. 2007) studied a population that lived in residences made with rebar that was contaminated with Cobalt-60 which made it highly radioactive. These studies suggested protective effects for cancer and birth defects. However, later studies of the same population using different epidemiological techniques did not demonstrate such a protection and suggested excess cancers in the population (Hwang et al. 2006).

Other human studies suggested an adaptive beneficial effect for chronic myeloid leukemia (Radivoyevitch et al. 2002) as well as other protective effects as a function of dose-rate (Leonard 2007b, a). These studies do not represent a complete review of the literature but provide directions for future research and direction.

A problem associated with protective effects of low doses of radiation has been the lack of understanding of the mechanisms involved. As the research in the low dose region continues, a mechanistic basis is being developed that helps to explain these observations (Schollnberger et al. 2002). Chapter 6 contains reviews of mechanistic data that impact the responses in the low dose region.

Application of all these low dose data to radiation protection standards is problematic and very difficult (Curtis et al. 2004). The impact of the Program on standards has been reviewed in detail. To date, the data have not had much impact on the standard setting process (Morgan 2006). With better mechanistic understanding and the use of systems biology approaches, more progress is expected in the future. This is discussed in more detail in Chapters 8 and 9.

The bottom line in much of this discussion is that many scientists question the LNT, and others call for its rejection as the basis of radiation protection standards; for example, (Cohen 2008), (Jaworowski 2008), and (Calabrese 2007). Part of the problem of rejecting the LNT is to determine what models and methods can replace it especially in setting standards. If thresholds exist, they seem to be different for each endpoint, tissue, organ and species, making threshold models almost impossible to use.

Hormesis has been suggested as the default dose-response model (Calabrese 2004), and for it has also been suggested that there has been a dose-response revolution supporting the rejection of LNT and replacement with hormesis (Calabrese & Baldwin 2003). Such a replacement suggests that there is no risk from low doses, and that low doses are beneficial and even essential. Such a change in the understanding of ionizing radiation effects in the low dose region is hard for many

in the radiation research community to accept and has been the subject of several debates and publications. Thus, there remains debate as to which models should be used to estimate radiation dose in the low dose region (Tubiana et al. 2008). This debate was triggered again by the release of two major reports on the health effects of low doses of radiation. One from the U.S. National Academy of Sciences (NRC 2006) supported the use of the LNT and produced a very impressive document to support their position (NRC 2005). The other report by the French Academy of Science (Tubiana 2005) reached the opposite conclusion and suggested that the LNT needs to be replaced. (Dauer et al. 2010) conducted a literature review and found that much new data has become available since BEIR VII that does not support LNT and that there remains a need to continue to evaluate the usefulness and accuracy of the LNT hypothesis.

IV. Low Dose Models

Using the LNT as the basis for cancer risk extrapolation has generated widespread concern. Using this approach and model has suggested that the risk for cancer in the future may be increased markedly by the use of medical radiation. Medical radiation exposures continue to increase, and at the current time it has been calculated that there are 70 million CT scans per year (Mettler 2011). The average dose to the bone marrow from a whole body CT can results in 10 mSv. By combining these two observations a collective dose that is larger than the natural background radiation can be estimated (Brenner & Elliston 2004; Brenner & Hall 2007) (Mettler 2011). With this approach 70 million persons receive CT scans that result in 0.01 Sv will result in700,000 person.Sv each year. From such a dose a large number of excess cancers can be calculated.

Compare this collective dose from the nuclear weapons fallout to that in the down-winders in southern Utah, where a population of about 25,000 people got an average dose of 0.03 Sv to result in a collective dose of 750 person.Sv. Even though the collective dose is low the congress passed the Radiation Exposure Compenstion Act (RECA). If any person develops a radiation-related cancer, such as the types of cancer that increased in the A-bomb population, lived in the Utah counties that had the highest fallout, and were in that county at the time of the fallout they get paid $50,000. To date, the RECA program has paid out more than a billion dollars in claims (Ziemer 2009). If a similar program were in place for those that received medical exposures it would result in payouts that would be so high as to be unacceptable. Similar comparisons can be derived from the nuclear workers. All this illustrates over-concern about the fallout and the nuclear workers. Because the dose is small from each CT scan, the risk is very small, and the real and immediate benefit from the scan is much larger than any calculated risk.

Using the LNT model, the extrapolated risk estimates, and the large collective dose there would be a very large number of calculated excess cancers (Brenner et al. 2001a; Brenner & Elliston 2004). Similar types of calculations were made for routine mammography screening (Brenner et al. 2002). What these calculations lack are the benefits derived from the exposures associated with the diagnosis of disease and a discussion of the potential protective effects of low doses and dose rate (Scott 2007). Including this information would help the public understand the tradeoffs they make when they get a medical procedure that involves radiation and help them make the informed choices associated with the procedures. (Brenner 2009) wrote a review that shows how

the extrapolation from low doses to very low doses is done and helps understand and justify the LNT procedure.

V. NASA Models

Research is being jointly funded by NASA and DOE to help understand the radiation risk associated with travel in space. Many of the goals of NASA and DOE are the same: to understand the influence of low dose and low dose-rate radiation exposure on risk. The differences are that the radiation environment in space is higher and different than that on the earth, with many different types of high Z energy particles (HZE) such as iron ions that have large mass and very large energies. Responses to these HZE particles may be unique because they deposit such a large amount of energy along the track that they travel in tissues. (Durante & Kronenberg 2005) wrote a useful review on HZE particle research and how this information could be used to evaluate space travel risk. Space also contains very large fluxes of protons that do not exist on the earth, and the average radiation dose rate is also much higher than that on earth. Studies of this higher dose-rate environment will aid in extrapolating the effects of higher dose-rates to the lower ones on earth. In the event of a terrorist attack or nuclear accident, dose-rates may be similar to those in space. This further defines the importance of studies conducted on the space radiation environment.

The radiation environment in space has been carefully defined and is measured on each space mission (NCRP 2010). To construct models appropriate for space flight, NASA is conducting a research program using appropriate types of radiation. The biological data collected from this program and data generated from astronaut evaluations are used to limit the uncertainities associated with the risk derived. The risks set for the astronauts are different than those set for workers on earth. Early modeling of the risk from space radiation was similar to that done for workers on earth. The initiation-promotion models widely used to model radiation effects were applied to space radiation to estimate the tumor prevalence from high charge and high-energy particles (Wilson et al. 1995). Other models such as the two-stage clonal expansion model (Curtis et al. 2002) that had wide application in defining cancer risk on earth were also applied to space irradiation.

Application of these models assumes that the initiation, promotion, and clonal expansion mechanisms responsible for cancer on earth is similar to those in space. However, as modeling and biological data have improved, the unique responses seen from space irradiation are being incorporated into the modeling efforts. (Cucinotta & Durante 2006) evaluated the cancer risks from the complex space environment including galactic cosmic rays, dose, and dose-rate. Such evaluations are essential to put space risk into the proper framework and minimize the uncertainity associated with these risk estimates.

Studies on the hematopoietic system are important because this is one of the most sensitive parts of the body to ionizing radiation. Studies using space-simulated photons (Gridley et al. 2008) as well as other types of radiation in a simulated solar particle event (acute exposure to photons and protons as well as solor particle event protons) were studied to evaluate their impact on cell killing, the immune system, and other responses associated with the hematopoietic system (Gridley et al. 2008). Additional research included bystander effects that may be produced by the

HZE particles in developing models of cancer risk (Brenner & Elliston 2001). Research and modeling studies continue to be funded by NASA and DOE to link the biological risks from space exposure to that in earth.

The debate over the use and appropriateness of the LNT continues (Tubiana et al. 2008) and has been showcased in several national and international scientific meetings. Many debates featured DR. David Brenner supporting the LNT, and he debated a variety of different scientists who were not in favor of the LNT (Brenner & Raabe 2001; Brenner & Mossman 2005; Averbeck 2009; Brenner 2009). One of the most productive debates was at the 2008 NCRP meeting. The debate featured a representative favoring the LNT and one against it (Averbeck 2009; Brenner 2009). The publications that resulted from this debate provided a useful update on the state of the science in this important area. Both sides suggest that they won the debate, but only time and additional science will tell (Tubiana et al. 2008). The problem with such debates is that the public doesn't know which side is "right," so the public perception associated with the risk from radiation exposure remains confused. As more data become available, the debate may generate more light and less heat.

VI. Animal Models

The use of molecular, cellular, and animal models has played an important role in understanding the mechanisms involved in radiation-induced cancer. Extensive research has been done on experimental animals to determine the dose-response relationships that exist between different types of radiation exposure and biological damage. These life-span studies will not be reviewed here. (Stannard et al. 1988) and (Thompson 1989) provide an extensive and useful review on the life-span studies conducted in the dog. These well-conducted studies provide an extensive data resource for comparisons to current research and to aid in extrapolation of radiation risk to humans. Extensive whole-life studies have also been conducted in rodents.[1]

With modern technology and the production of mice with specific genetic backgrounds, the development of knock-in and knock-out mice that contain known genes of interest provides great research potential, and these are not be reviewed here. A resource for genetically defined animals is available at ORNL and funded by the Program. These recombomice are very useful in studies to evaluate the role of genetic background on responses to radiation.[2] It has been suggested that *in vivo* recombination following chronic exposure in these mice is decreased below the spontaneous level as a form of adaptive response (Kovalchuk et al. 2004). (Hendricks & Engelward 2004) wrote a useful review on these recombomice that defines how they are derived and many of the potential uses in scientific studies (Hendricks & Engelward 2004).

Animal models have been developed that can be used to study specific diseases; for example, a mouse strain is available that can be used to study radiation-induced AML (Darakhshan et al. 2006). Another model is available for study of Nijmegen syndrome (Williams et al. 2002). Many such mouse models provide a genetic background that is either sensitive or resistant to radiation-

[1]To get information or tissues from the life-span studies on rodents, contact Dr. Gayle Woloschak at g-woloshack@northwestern.edu

[2] To get information on the mice, contact Dr.Brynn Voy at voybh@ornl.gov

induced cancer. These models have been exposed to low doses and dose rates, have demonstrated marked adaptive responses, and suggest that low doses might be protective from radiation-induced cancer (Mitchel 2006).

VII. Molecular, Cellular, and Tissue Biology Models

The basic mechanistic data on radiation effects in the low dose region was reviewed in Chapter 6. This information was used to develop models at the molecular, cellular, and tissue levels and to suggest as to how such data might be applied to cancer risk assessment.

A. Modeling DNA Damage

In developing models it is important to evaluate the changes on the basic level of the DNA and to understand how radiation interacts at this level to produce DNA damage and repair. There was a dose-dependent mis-repair of DNA double strand breaks which was modled for both high and low-LET radiation (Rydberg et al. 2005). The type of radiation exposure is important in evaluation of such damage as was seen in the models of space and microbeam radiation. The role of energetic electrons (100 eV to 100 keV) were studied and models developed to predict the type of DNA damage produced (Nikjoo et al. 2002).

An observation made about radiation-induced DNA damage was that multiple damage sites occurred in a small area. Methods were discussed and results of research conducted on fragment and multiply damaged sites in the DNA in Chapters 5 and 6 (Sutherland et al. 2001b; Sutherland et al. 2001d; Sutherland et al. 2003a; Sutherland et al. 2003b). The size of the DNA fragments was also important because from the size distribution, it was possible to develop models that could predict the different levels of structure in the DNA. Models of DNA breakage based on random walk, a mathematical formalization of a trajectory that consists of taking successive random steps, were developed to understand how chromatin structure influenced the DNA breakage (Ponomarev et al. 2001a). Other models of DNA breakage following high doses of radiation were used to predict the damage induced by low radiation doses (Ponomarev et al. 2001b). Such models will be useful in making the extrapolations between high and low doses of radiation and can help test the shape of the dose-respone curves.

These models were used to study the influence of the LET of radiation to help understand DNA breakage induced by HZE particles in space (Ponomarev et al. 2001a). This again provided preliminary information that can be used in risk estimates following exposure to the space radiation environment. In other research on space radiation, DNA DSBs were produced by nitrogen ions with a wide range of LETs. The model predictions for the induction of clusters of DNA DSBs were compared to the measured frequency and distribution to validate the model (Fakir et al. 2006).

Other studies were conducted using a Monte Carlo algorithm to simulate the spectrum of DNA damage induced by ionizing radiation (Semenenko & Stewart 2004). This research was followed up by studies to evaluate the repair of the clustered DNA damage sites. Repair of these lesions is thought to be limited and may be important during the development of cancer. This study evaluated both base and nucleotide excision repair of these lesions and matched the repair to the model properties (Semenenko & Stewart 2005). In the companion paper (Semenenko et al.

2005), this research was expanded, and the measured DNA repair was compared to that predicted with good results. Such studies as these are essential to gain confidence that the model will predict the real world.

One of the concerns about this research was the potential to produce DNA breakage during the processing of the samples and the difficulty in determining the radiation-induced DNA breakage versus that produced as an artifact. Methods were developed to solve this problem and reduce the breakage produced during the preparation of the samples (Ponomarev et al. 2006). With these new techniques in place studies additional studies will be needed to determine the role of high to low dose extrapolation, the influence on chromatin structure, and the influence of HZE particles in producing DNA damage.

As discussed in Chapter 6, DNA damage acts as a trigger for many changes in the cells, and radiation affects the change in gene expression. Model methods have been derived to detect changes in gene expression in the presence of inter-individual variability (Rocke et al. 2005), and these need to be applied widely in the studies described in Chapter 6.

B. Modeling Chromosome Aberrations

The next level of cell and molecular organization thought to be important in radiation-induced cancer is chromosome aberrations. Many cancers have well-defined chromosome aberrations, some of which seem to be markers of the radiation-induced disease. Studies and development of models of chromosome aberrations provide a useful foundation for radiation related cancer risk. With the development of chromosome painting techniques described in Chapter 4 it has been possible to identify every chromosome and determine which chromosomes interact to form radiation-induced breaks. These techniques were combined with DNA damage processing pathways, which made it possible to link DNA damage to the production of chromosome aberrations (Levy et al. 2004). Such links between different levels of biological organization form the basis for future modeling using systems biological techniques to be described in more detail in Chapter 8.

Several models were developed to predict chromosome aberration frequency and interaction distances between chromosomes. One of these was the random breakage and reunion model, which suggested that all interactions between chromosomes were random, and the chance of any chromosome interacting with any other was only dependent on its size. With such a model it was possible to predict interaction distances based on chromatin geometry (Sachs et al. 2000). The development and further application of chromosome painting techniques made it possible to stain interphase cells and determine the domain of the chromosomes during interphase. Such information can be linked to the production of aberrations that are expressed when the cells have progress to metaphase stage of the cell cyle.

Using chromosome domain during interphase models were developed to predict radiation-induced chromosome aberrations (Holley et al. 2002). It became possible to generate chromosome aberration spectra and use them to predict aberration frequency and interactions between chromosomes (Levy et al. 2007). Before chromosome painting was available it was thought that all the chromosomes interacted with each other randomly, and the frequency of this interaction was only dependent on the chromosome size. With additional analysis and modeling

it was determined that there was an excess of radiation-induced chromosome aberrations between homologous chromosomes (Plan et al. 2005). This suggested that the location of the chromosome in the interphase nucleus played an important role in the induction of chromosome aberrtions.

As additional data was generated using chromosome-painting techniques it was demonstrated that there are cells that contain very complex chromosome aberrations with multiple chromosomes involved in the aberrations (Vazquez et al. 2002). Such aberrations were difficult to explain based on older models. New methods of biophysical modeling were put in place, and additional insights were gained on how these complex chromosome aberrations could be formed (Hlatky et al. 2002; Sachs et al. 2002). These models were further developed and produced quantitative analyses of radiation-induced chromosome aberrations that were related to the observed number and types of aberrations (Sachs et al. 2004).

Another measure of chromosome damage is the induction of micronuclei. These represent small pieces of chromosome that are not included in the nucleus after cell division. They are easy to score and can be used to relate physical variables to the induction of chromosome damage. One of the mechanistic studies done with micronuclei was to set up culture dishes in a way that energetic heavy ions would traverse the cells with different portions of the ion track being located in different parts of the culture system. With this system it was postulated that it would be possible to detect the influence of the Bragg peak, where there are more ionizations per unit distance traveled, on the induction of micronuclei. There was a suggestion of an increase in this area but it was not as great as would be predicted based on the number of ionizations deposited in that region of the dish and in those cells in the region (Wu et al. 2006). Such studies support the concept that all the cells in the culture dish are responding to the insult, and the bystander effects may be influencing the total response.

C. Modeling Cell Killing

It is well established that radiation kills cells effectively and is used in therapy because of this characteristic. Models have been developed to describe radiation-induced cell killing. The induction of DNA damage and the failure of that damage to repair have been thought to be a source of cell killing. Two-lesion kinetic models were developed to determine if rejoining of DNA DSBs was directly linked to cell killing (Stewart 2001). These models predicted an association between these two biological processes but suggested that other mechanisms for cell killing are involved following exposure to ionizing radiation. Microdosimetric models were linked to cell killing through bystander effects. The type of bystander effects studied were those that were transferred through the media and were not dependent on cell-cell contact. It was demonstrated that the response could be explained using these microdosimetric models and that this interaction with the cells was responsible for the release of soluable substances into the media (Stewart et al. 2006).

The cell cycle and the length of time that cells spend in each part of the cycle varies depending on the tissue. Most epithelial tissues, which are the source of radiation-induced carcinomas such as in the liver, have most cells in the G_o or resting phase of the cell cycle. When an insult kills cells, other cells must divide and replace them. This stimulus for cell proliferation plays an important role in radiation-induced cancer, especially following high radiation doses. In

developing models for carcinogenesis it is important to include consideration of the cell cycle and the movement of cells from one stage of the cycle to the next. A multistage carcinogenensis model was developed that used cell cycle as one of the variables, which is a great step forward in modeling (Hazelton et al. 2006). Movement from the G_o to the other stages of the cycle may be one of the triggering events in radiation-induced cancer.

Most studies on cell killing have been conducted in tissue culture systems using *in vitro* single-layer cell cultures. Extensive cell-cell interaction can modify the killing of mammalian cells. Studies using a vertebrate embryo were very important in explaining many of these interactions (Bladen et al. 2007b). Such studies also support the role of bystander cells in protecting and modifying the responses to ionizing radiation.

D. Modeling Bystander Effects

The data from bystander effects have been modeled to understand the role of this observation on radiation-induced cancer risk. Because DNA DSBs have been shown to be important in producting chromosome aberrations and have been linked to cancer induction, it is important to determine if DNA DSBs are induced in bystander cells that have no direct energy deposited in them. Using a microbeam, (Sedelnikova et al. 2007) determined that DNA DSBs could be produced in cells that do not have energy deposited in them. These studies were carried out in a three-dimensional human tissue model and support the theory that bystander effects can be detrimental the cells and organisms.

An early model of bystander effects combined the damage from the Bystander Effects and Direct effects (BaD) model developed by (Brenner & Sachs 2002a). Using this model, it was suggested that bystander effects could potentially dominate radon risk (Brenner & Sachs 2002a, b). Additional data was published that suggested that the damage from bystander may be significant but the risk from radon would not be influenced or changed significantly by the bystander effects (Brenner & Sachs 2002b, a, 2003). Other models have been generated suggesting that bystander effects can be either detrimental or protective (Schollnberger et al. 2007). The adaptive response has been demonstrated in bystander cells, and after low doses of radiation many protective mechanisms have been shown to be triggered in bystander cells. There is little doubt that bystander effects exist following radiation, and the effects of this were again demonstrated to potentially be beneficial (Azzam & Little 2004). The review written by (Ballarini et al. 2002) is useful but requires constant updating as additional data are accumulated.

The bystander effects are the result of cell-cell and cell/matrix communication. Modeling this communication both in terms of cancer formation and radiation-induced damage has been very useful. Some models have focused on cell cultures and tried to get a comprehensive stochastic model of these cultures (Hanin et al. 2006). However, it is well established that cells in monolayers in culture do not respond to radiation in the same way that cells grown in three-dimensional cultures do. Modeling engineered cultures of breast demonstrated that the architecture, function, and neoplastic transformation is very dependent on the culture conditions and the interaction of the cells in three dimensions (Nelson & Bissell 2005). Special models of intercellular interactions that are essential in the cancer process further support the role of bystanders and communication in both increasing and decreasing the cancer frequency following radiation or other environmental insults (Sachs et al. 2005). Many of these interactions that

modify the ultimate outcome of the exposure seem be be dependent on gap junction communication (Green et al. 2005), and blockers of gap junctions such as Connexin 32 can eliminate the bystander effects in many cell culture systems (Green et al. 2002).

E. Modeling Genetic Background

The genetic background of any biological system has a marked influence of the response to radiation. This fact needs to be further evaluated as research on the risk and modeling of this risk proceed. For example, biologically based modeling of chronic myeloid leukemia provides a path forward for modeling many other diseases that are influenced by genetic background (Radivoyevitch et al. 2001). The influence of genetic background on risk has recently been reviewed (NCRP 2010). This document demonstrates that genetic background is important but currently, the tests for identification individuals that are at increased risk are inadequate to be applied or impact radiation protection. Several human genes are known to increase the risk for radiation-induced cancer. For example, BRAC 1 and 2 increase breast cancer risk. A review of the data on genetic background on risk is provided in the NCRP reference for those interested in follow-up of this subject.

VIII. Risk Assessment Models

A major goal of the Program was to provide a large, well-documented database on the effects of radiation in the low dose region. Using this database, reviewed here, will make it possible to develop a scientific basis for risk assessment and to justify and identify the models that are used to predict risk in the low dose region (Brooks 2000a, 2003). As a more systems approach is taken to understanding carcinogenesis, the role of genetic background and cell-cell and cell/matrix communications must be considered, because they play an important role in promoting or inhabiting cancer development.

Major Points: Modeling

- Traditional models were used to fit human epidemiology data.
- In the higher dose ranges both linear and non-linear models have been used to fit the human data.
- In the low dose range the biophysics of energy deposition and “hit” theory have dominated the field of radiation biology and resulted in LNT hypothesis and models.
- LNT models are essential in controlling radiation exposure but may not be an accurate reflection of radiation risk.
- Extensive data has been generated and modeled that incorporate biological data at all levels of biological organization.
- Models suggest mechanisms of action that can be tested by experimentation.
- Many molecular, subcellular, cellular and animal models have non-linear dose-response relationships suggesting different mechanisms of action for the production of damage in the low dose region compared to the high dose region.

- Models of DNA and chromosome damage have been very helpful in understanding how the damage is formed and repaired.
- Models of data that demonstrated a decrease of background levels of damage by low-doses of radiation suggest the potential for adaptive and protective effect in the low dose region.
- Models of cancer must represent all the mechanisms and molecules involved in radiation induced initiation, promotion and progression of cancer.
- The continued use of biological based models has played an important role in the evaluation of the data generated by the Program.
- LNT models are adequate in the high dose region of the dose-response relationships but do not fit the cell and molecular data in the low dose region.
- Special models have been developed to evaluate cell and molecular responses.
- The influence of radiation type on models has played an important role in the development of models for the risk associated with space flight. These models must consider exposure to HZE particles (very high mass and energy particles) that are encountered in space.
- Animal models continue to play a central role in transferring information from basic science to epidemiology.
- Many animals model with defined genetic background can be used in radiation studies that provide increased mechanistic understanding when linked to molecular, cellular and tissue models.
- Because radiation is a very good cell killer, models of killing have been useful in radiation therapy.

Chapter 8

Taking a Systems Biology Approach to Risk

As described in the previous chapters, extensive datasets have been generated on the effects of low doses of radiation. The new technology and techniques developed or applied by the DOE Low Dose Radiation Research Program have been important in defining the biological responses in the low dose regions. These extensive datasets have resulted in better understanding of the mechanisms of action for radiation in the low dose region. They have also resulted in some unique models of radiation-induced biological changes.

Interactions between the many different mechanisms of action present following exposure to low doses of radiation and the modeling of these responses are complex. The data generated in the Program were a major factor in defining and recognizing these complex responses to low doses of radiation. The observed multiple responses and complex interactions require a new approach to modeling the responses in the low dose region. The old approach was to assume that DNA damage was the mechanism of action for damage from radiation in the low dose region and linear models were used to describe damage and risk. Ultimately it will be essential to extrapolate responses across different levels of biological organization (Feinendegen et al. 2007) to determine the shape of the dose-response relationships in the low dose region. The dose-response relationships can then be applied to estimate the risk associated with low doses of ionizing radiation.

One new approach to understanding the biological responses in the low dose region has been described as systems biology. Systems biology integrates the responses from the molecular to human population studies into complex models. These models are then used to determine how biological responses influence risk at each level of biological organization and are combined as these unique levels of biological organization are reached. This systems approach is illustrated in Table 1, which shows that studies from all levels of biological organization need to be linked. The ultimate goal is to develop a level of understanding of the mechanisms of action of low doses of radiation that makes it possible to predict responses and to use these responses to move up to the next level of complexity. When it is possible to predict responses it will also be possible to define the shape of the dose-response relationships and appropriately associate the risks with these low dose exposures.

Differences between High- and Low-Dose Radiation Responses	
High Dose > 0.2 Sv	**Low Dose < 0.2 Sv**
Cell killing high	Cell killing low
DNA damage high	DNA damage low/not detected
Gene Expression	Gene Expression (Protective?)
Epigenetic Effects?	Epigenetic Effects (Protective)
Free Radical Increased	Free Radicals decreased
Direct Action	Indirect Action ↑ MnSOD ↑ Glutathione
↑ Apoptosis	↑ Selective Apoptosis
↑ Mutation Frequency	↓ Mutation Frequency
↑ Cell Transformation	↓ Cell Transformation
Immune response (-)	Immune response?(+)
Cancer increased (5%/Sv)	Cancer (mSv)?

TABLE 1. Comparison of mechanisms for cancer induction at high and low doses of ionizing radiation.

One of the driving forces in development of a systems biology approach was the recognition that DNA damage is not the only important target in production of cancer (Barcellos-Hoff 2005a). The damage also triggers a set of signaling processes that plays an important role in radiation-induced cancer (Barcellos-Hoff 2005b).

The ability to measure changes in gene expression in large numbers of genes made it possible to generate very large datasets that describe radiation-induced changes in gene expression. These data demonstrate that gene expression changes as a function of many different exposure, physical, and biological parameters. The publications on these large datasets generated on radiation-induced changes in gene expression were discussed in Chapters 5 and 6. Such information illustrates that changes in gene expression provide a functional genomics approach and entry into systems level biological studies (Amundson et al. 2008). Such systems biology genomics research provides one of the basic sets of data needed to determine the responses of biological systems to low doses of radiation. However, with the generation of such massive data sets, new methods are required to analyze these databases. Several of these methods to handle data, such nearest neighbor analysis, cluster analysis, self-organizing maps, and computational

methods to evaluate degree and significance of increase or decrease in expression were reviewed in Chapter 5 and 6.

The level of change in gene expression does not link directly to changes in protein expression and function, which makes it essential to evaluate radiation-induced changes in protein levels, modifications, and activity. New technologies have used shared peptides in the quantification of different proteins. This label-free technique uses a combination of liquid chromatography/tandem mass spectrometry (LC-MS/MS) to determine the proteins expressed and the levels of those proteins (Jin et al. 2008). Identifying large numbers of protein changes makes a systems approach to evaluating these data and linking them to changes in gene expression essential.

Other technologies were also developed to identify protein modifications, especially phosphorylation and protein localization in the cells. The protein modification was measured using a linear discriminant analysis to accurately identifiy the modified phosphopeptides (Du et al. 2008). Studies that determine the impact of protein localization on biological function were also developed (Raman et al. 2007). Both phosphorylation and protein localization alter cell and tissue function and must also be considered in systems biology approaches.

Each of these cell and molecular assays must be linked to a functional measure that is important to radiation-induced cancer. Without such links the molecular data are not useful in evaluating radiation risks. A study by (Miller et al. 2008) relating changes in proteins to genomic instability illustrates how changes at the molecular level can be related to functional changes in the stability of the genome. In this study, changes in mitochondrial proteins were defined in genomically unstable cell lines, and the protein changes were related to the oxidative status of the cells and the maintainance of genomic instability. Such studies provide potential methods to extrapolate from genomic instability to oxidative status of the cells and to relate the ROS status of the cells to cell transformation and ultimately to radiation risk. Many steps and much information are required to make these links, but this illustrates how systems biology can be used in radiation risk estimates.

In Chapter 6 the interactions between cell and matrix were described. It was determined that exposure of the mammary gland stroma promotes the formation of tumors in unirradiated epithelial cells (Barcellos-Hoff & Ravani 2000). Cell-cell and cell-matrix interactions and signaling are involved in formation of cancer. The interaction of low doses of radiation with the microenvironment suggest new mechanisms of action that become an important part of understanding risk and systems biology (Tsai et al. 2005). These observations and approaches are useful in describing multicellular interactions and how they can modify outcome and perhaps risk. It has also been suggested that similar approaches may be used to evaluate radiation-induced multi-generational responses (Barcellos-Hoff & Costes 2006).

It is important to be able to describe signaling pathways, understand the biological modifications associated with the signaling, and generate models that adequately describe the pathways. As signaling pathways have been described it became evident that certain “nodes” of activity exist where single proteins or genes play critical roles in controlling multiple pathways. An illustration

of this type of interaction is shown in Figure 20. Using multiple types of genomic approaches provides more accurate data on the mechanism of action.

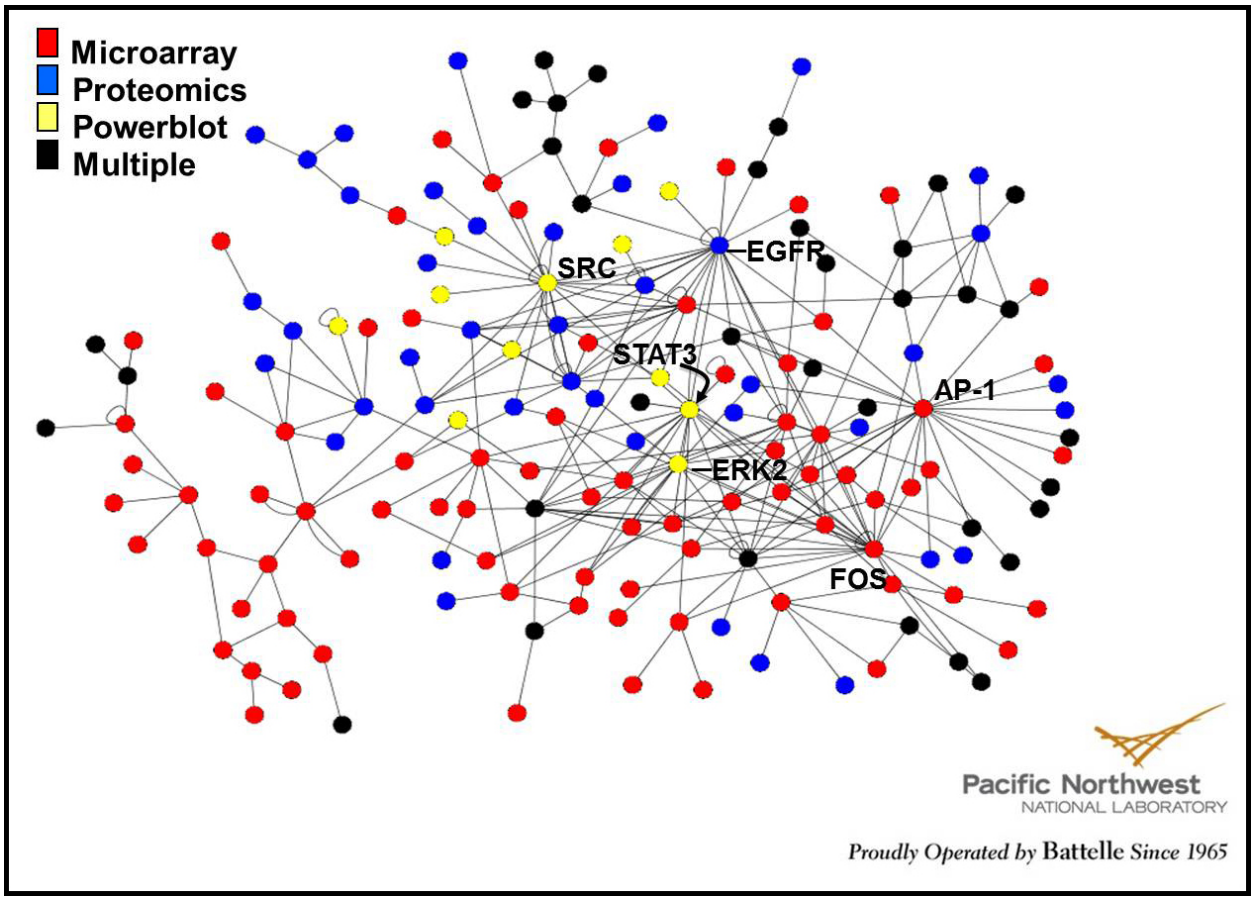


Figure 20. Integrated data provide more comprehensive and accurate network reconstruction.

The communication between all these pathways becomes very complex. Important advances in data analysis involved in signaling pathways and how they can be modeled have been made (Miller & Zheng 2004). Such evaluations represent one of the key elements of systems biology and will be used more widely as additional data are provided on the mechanisms of cell-cell communication and the interactions that control the expression of cancer. (Petrini 2007) reviewed the role of cell and matrix communication and showed that the interactions between cells are critical to the cellular function.

The research that generated data demonstrating the phenomena of bystander effects, genomic instability, and adaptive response suggest that whole tissues are responding to radiation exposure. Such tissue and organism responses to radiation imply that radiation-induced cancer is an emergent phenomenon and that many complex processes are involved at all different levels of biological organization (Barcellos-Hoff 2008). These processes seem to change as a function of radiation dose with high doses initiating a different set of processes than low doses.

It was recently shown that using a systems biology approach, it is possible to make useful risk estimates for astronauts that are exposed to a wide range of different types of radiation (Cucinotta 2008). Such modeling exercises provide guidance that will be useful in determining the slope of the dose-response relationships and the radiation-related risks in the low dose region. National and international meetings have helped provide future direction for research in the area of systems biology. As additional research is conducted and integrated improvement of the role of low doses of radiation on risk estimates can be improved and the uncertainity associated with these risk estimates reduced.

Major Points: Systems Biology

- Many biological changes and mechanisms associated with risk from ionizing radiation in the low dose region were explored by the Program. Systems biology approachs enable all of the new data to be considered and related to risk.
- The Program determined that systems biology was a good approach to integrating many of the new findings for the following reasons:
- Simple models of DNA damage and response do not adequately describe all the complex biology that is involved in responses to low doses of radiation.
- Models need to be constructed at each level of biological organization. These models can then be link across levels so that mechanistic molecular and cellular models are related to well-defined functional changes.
- Coordination of research projects focused on useful models were used to relate molecular and cellular changes to functional endpoints as an intermediate step in predicting radiation risks in the low dose region.
- The very large data bases were generated on the biological responses induced by low doses of radiation (changes in gene expression, the epigenome, and proteins) required a systems biology approach to integrate them into risk assessment models.
- The Program attempted to relate functional changes at the cell and tissue level to cancer risk to be useful in risk estimates.
- Additional research and efforts are needed to incorporate Systems Biology approaches into risk estimates.

Chapter 9

Program Communication and Monitoring

A major goal of the DOE Low Dose Research Program was to monitor and communicate the results of the research effectively, first to the scientists involved in the research, then to policy makers to help government agencies use the information to set standards to control radiation exposure, and third, to stakeholders and the public to help them make informed choices associated with the risks from radiation. During formation of the Program the BERAC subcommittee outlined the key question related to communication, "How can the information derived from the low-dose initiative be best communicated to scientists, policy makers, stakeholders and to the public?" They further suggested methods and topics to be included in the research plan to accomplish this:

"To communicate with the public about low dose management requires a well-developed plan based on strong basic social science research. The goal of research effort would be to understand the likely public responses to scientific findings from the Low Dose Program's research and responses to the plans for modifying existing standards based upon these scientific findings. The following topics should be included in determining public responses to issues regarding low dose exposures: 1) public perceptions of risk; 2) the perceived importance of the activities and conditions that produce low dose radiation; 3) trust and confidence in risk managers, regulators, and decision makers; 4) the role of the media in characterizing different positions on risk controversies; 5) the role of advocacy groups; 6) the manner by which risk is characterized and assessed; and 7) the procedures by which decisions are made."

With this in mind, several approaches and research projects were funded to carry out this part of the research plan.

I. Advisory Committee

At the start of the Program a science advisory committee was organized as a subcommittee of BERAC by Dr. David Thomassen who was the Program Coordinatory, Office of Biological and Environmental Research to aid in the communication and direction of the Program. The Committee was chaired by Dr. Sharon Friedman, Lehigh University. The Committee provided valuable guidance during the Program's early development and was instrumental in getting funding to help communicate research results and set up mechanisms to keep this communication active.

II. Workshop on Risk Communication

In 2000, a workshop was held at Decision Research Institute in Eugene, Oregon, titled "Workshop on Low Dose Radiation Exposure and Risk Communication," which was organized by Dr. David Thomassen and was under the direction of Drs. James Flynn and Paul Slovic. In this workshop the communication needs and the needs and problems associated with risk

communication were carefully reviewed and discussed. The complete results of this workshop can be found in a report to the Department of Energy, Office of Science, Office of Biological and Environmental Research. The topics covered included:

- Underlying Problems of Risk Communication
- The Social-Cultural Context
- Organizations, Institutions, Trust and Risk
- Guides to Study the Social-Cultural Context for Risk communication
- The Social Amplification Risk Framework
- The Social Geography of Risk Communication
- Value-Bases Structured Decision Processes
- Cross Cutting Research Questions: What should we attempt to learn from our studies?
- Research Tasks: Studies to improve risk communication within its societal context
- Conclusions: The next steps for the risk communication research project

This workshop addressed a number of communication problems that would come up repeatedly during the Program and provided a backdrop against which DOE could help address these problems. Additional meetings were held with those involved in communication, including the media, who suggested that it would be difficult to get this information into the public because much of it was not considered "newsworthy" by the media. Providing the public information that would change the way that they perceive radiation risk is essential, but such communication may be very difficult because of the media's lack of interest in positive information about the low risk associated with radiation exposure (Flynn & MacGregor 2003).

This has proven to be true as the Program has progressed. Only research that tends to raise alarm about radiation is released by the media. Research data suggesting that scientists understand the risks of low doses of radiation, that the risks are well defined, and that radiation risks may be adequately conservative are very difficult to get into the media and placed before the public. This remains a major challenge of the Program because the public and regulators have models and hypotheses that they accept and are unwilling to expand their vision beyond these (Leonard 2008).

III. Lead Scientist

The first DOE call for proposals in 1998 requested applications for a Lead Scientist to work closely with DOE in facilitating Program operations. Among the requirements for the lead scientist: "The Lead Scientist be funded from the program and will provide scientific leadership to the community of the researchers in the research program...Interested applicants should demonstrate their understanding of the needs for and the uses of the types of scientific information likely to be developed in this research program. They should demonstrate their understanding of previous epidemiologic and experimental studies involving low dose, low dose-rate exposures to radiation or chemicals. Finally, interested applicants should demonstrate their knowledgeability of research opportunities and capabilities at National Laboratories, universities,

and industry in the area of molecular and cellular responses to low dose, low dose-rate exposures." Dr. Antone L. Brooks, from Washington State University, was selected as the lead scientist and served in this position until 2004, when he was succeeded by Dr. Mary Helen Barcellos-Hoff, Professor of Radiation Oncology and Cell Biology at NYU Langone Medical Center, New York.

IV. Investigator Workshops

A key venue for communicating research results is the annual Investigator Workshop. All principal investigators funded by the Program are required to participate in these workshops by presenting a poster summarizing their scientific progress over the past year. DOE has striven to organize a highly focused symposium on a single theme or issue, in which the current state of the art is reviewed, and potential future directions assessed.

Individual scientists who have made important contributions to the Program are asked to make oral presentations that further elaborate on their progress and the general workshop theme. In addition, DOE invited scientists from outside the Program who have made important scientific breakthroughs in the field to make presentations. These presentations help keep the Program scientists up to date on the research progress of the field. Interactions between the Program scientists and these invited experts are vital in developing new scientific direction for the Program. Finally, the workshop provides the opportunity for interactions among the Program scientists that can lead to collaborations that can become the basis of future research and help guide the Program's direction.

Workshop participants include BER program staff, program staff at other agencies, BERAC subcommittee members, and scientists from other DOE-funded programs whose research has useful links to the Program. In addition, staff from regulatory agencies, such as the EPA and the NRC, actively participated.

Earlier in the Program, extensive efforts were made to invite members of the public and political action groups with interest in radiation issues. At these early meetings, members of Indian Tribes, Hanford Advisory Board, the Mayor of Rocky Flats, Colorado, political action groups, downwinders, and nuclear workers with interests and concerns about exposures to low doses of radiation attended. These workshops included small discussion groups, with the members of these public groups placed in groups with scientists and regulatory agency representatives to address mutual concerns. These workshops helped develop understanding and trust among the participants. As the invitees got to know the scientists, they learned that the researchers did not have an agenda and that the research produced would have a firm scientific basis. In later years, the workshops have become more focused on the scientific issues and future directions, but these groups continue to be invited and still participate actively during the presentations and discussions.

V. Presentations

As Chief Scientist Dr. Brooks gave 76 presentations to the radiation scientific community between 1998 and 2008 to ensure it was aware of the Program and its research. He also made

presentations to groups outside the radiation community, such as the American Chemical Society, American Pharmacists Association, American Statistical Association, International Consortium for research on health effects of radiation (ICRHER), DOE National Laboratories, the Washington State Department of Ecology, universities, research laboratories, and agencies like RiskRad that fund low dose research in Europe. Presentations were also made to U.S. government agencies, including DOE Washington DE, DOE Richland, EPA, NIH, and NIAID.

The Program Managers also gave presentations to other agencies using data generated from the Program to help these agencies understand the Program's importance. They also made presentations to agencies and institutions involved in making recommendations related to radiation standards, such as National Council on Radiation protection and Measurements (NCRP), Electric Power Research Institute (EPRI) and International Council on Radiation Units (ICRU), as well as to BEIR VII of the National Academy of Sciences. These are discussed in more detail in Chapter 10.

It was also important to make presentations to the public to help with the understanding of the magnitude and importance of the program. There were 37 presentations made to different groups like Rotary, Nez Perce Tribe, Navajo Tribe, Down Winders in St. George Utah, Hanford Advisory Group, Boy Scouts of America, and many other public groups. Power point representations of these presentations are available by contacting Dr. Antone L. Brooks.

VI. Website

The major source of communication of the Program's research results to the public and researchers both in and outside the Program was its website. The website was originally developed at Oak Ridge National Laboratory under the direction of Dr. John Wassam. Lead scientist Dr. Antone Brooks developed the background information addressing the public's needs and following the scientific progress of the Program. The website was transferred to Washington State University-Tri-Cities in 2001, under Dr. Brooks' management. When Dr. Brooks retired in 2008 the website was moved to the Pacific Northwest National Laboratory under the direction of Dr. William Morgan. The website is at http://lowdose.energy.gov, and it contains dose rate charts, research highlights, a database of publications that have been produced by the Program, Program project descriptions, frequently asked questions, a glossary of radiation-related terms, an inquiry page, and links to other radiation research programs and resources. This site continues to be an important method of communication with the public.

VII. Open Literature Publications on Communications

Program-funded research resulted in many publications in the open literature that followed on previous research on the public's perception of risk from radiation. It is well established that many myths and stories have resulted in a high level of fear of the health effects of radiation exposure among the general public (Slovic 1996). Results of studies of how this fear was generated suggested that the assignment of numbers to risk and the concept that every radiation induced ionization increases cancer risk have been major contributors (Purchase & Slovic 1999).

In the field of radiation biology the questions asked about low dose exposure have been “How low is low enough? What dose is acceptable?” These are the same questions asked about any environmental exposure to chemical or physical agents. Early publications from the Program were focused on the question asked by (MacGregor et al. 1999) in their article “How exposed is exposed enough? Lay inferences about chemical exposures”. This kind of information is essential as a basis for public discussion and education and to understand how the public views radiation (Flynn & MacGregor 2003; Leonard 2008). Such questions are critical in helping regulators and the public understand the role of low doses of exposure on risk induced radiation or any other toxic agent.

A number of open literature publications about the Program’s progress were generated and put into a number of different scientific journals and proceedings (Brooks 2000a, b, c). Also published were papers describing how the results of the program would provide a scientific basis for standards (Brooks 2003; Brooks et al. 2007).

Major Points: Communication and Monitoring

- Communication of research results was and remains a priority of the Low Dose Program. This included communication between researchers, communication of the data to regulatory agencies, and communication and education of the findings to the public.
- An advisory committee was set up to provide input and direction for the program and a lead scientist was funded to provide a vital link between the Program and the scientific community.
- The Program emphasized open literature publication of the data generated in the Program to provide public and scientific access to this information.
- The annual contractors meetings provide the opportunity for all involved in the program to interact and gain valuable information from interactions with others in the program.
- The Web Site has become the major repository for information on the health effects of low doses of radiation, a site to store and access data published in the open literature, and an educational resource for the general public on radiation exposure, doses and risk.

Chapter 10

Current and Potential Impact on Standards

I. Standards Setting in the Low Dose Region

In the late 1990s when the Program was first being funded, there were many ongoing activities associated with radiation standards. The research associated with the Program had substantial input into these. From the start of the Program, it was important that the data be recognized and used as part of the process in determining the risk from exposure to low doses of ionizing radiation, and efforts have been made at every step to do this (Brooks 2003). From 2005-2008 four reports were published that have direct impact on radiation standards.

In 2006 the National Academy of Sciences published the Biological Effects of Ionizing radiation (NRC 2006) report. Dr. Brooks made a presentation early in the preparation of this report (October 2000) that outlined the research projects being funded by the DOE Program and discussed the potential impact and future data that would be available. The BEIR VII report has a good review of Program data generated from 1999 to 2004. As pointed out earlier, the BEIR VII report suggested that while important, the data on bystander effects, adaptive responses and genomic instability were not developed adequately. It would require a better mechanistic understanding of these processes for them to be useful and be included in the standard setting policy.

BEIR VII continued to use the biophysical LNT model for extrapolation of risk into the low dose region where it is not possible to gain useful information from human epidemiological studies. With this model the risk of cancer proceeds in a linear fashion at lower doses without a threshold. Thus, according to BEIR VII, the smallest dose of radiation has the potential to cause a small increase in cancer risk in humans. The Program's results, however, have demonstrated that there are very different biological responses following low doses of radiation than those observed after high radiation doses. Thus, the mechanisms of action in the low dose region are different than in the high dose. There is a well-defined transition in responses in the dose range of about 0.1-0.2 Gy where the slope of the line is lower than observed in the higher dose region. The challenge remains to determine if the slope is zero, greater than zero, or less than zero in this low dose region.

In 2009 the Electric Power Research Institute funded a review of more than 200 publications published after the BEIR VII report (Dauer et al. 2010). This review demonstrated that additional mechanistic data were available on the responses of biological systems to low doses of radiation that need to be considered in future standard setting activities. The report supported the need for a low dose rate effectiveness factor and reviewed the evidence that the mechanisms of action change as a function of dose and that the slope of the dose-response curve in the low dose region is lower than that observed in the high dose region. A summary of this report was recently published (Dauer et al. 2010). These data suggest that the current standards are adequately conservative when the LNT is used to predict the risk in this region.

Two parts of a 5-part report have been released by The United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR). The report includes five scientific annexes, the last three of which have not yet been released:

1. Epidemiological studies of radiation and cancer
2. Epidemiological evaluation of cardiovascular disease and other diseases following radiation exposure
3. Non-targeted and delayed effects of exposure to ionizing radiation
4. Effects of ionizing radiation on the immune system
5. Sources-to-effects assessment for radon in homes and workplace

They concluded from the report that the risks from cancer and genetic effects previously recommended did not require any change at this time.

Additional reports were issued by the ICRP. Report 99 by (Valentin 2006) evaluated the low-dose extrapolation of radiation-related cancer risks. Report 103 update dradiation protection recommendations. This report suggested the continued use of the LNT hypothesis combined with an uncertain dose-dose rate effectiveness factor (DDREF) to extrapolate risks from high doses of radiation into the low dose region.

Finally, a report from the French Academy of Sciences raised some serious questions about the validity of using the LNT for evaluating carcinogenic risk in the low dose region. They suggested, as has been supported by the Program, that the biological mechanisms and responses are different at low doses and high doses. They suggested that the use of the LNT model may lead to an overestimation of risk at low doses.

After organizations such as the NAS, NCRP, and ICRP make recommendations, it is up to the government agencies charged with controlling exposure to determine how to use them. Dr. Brooks was a member of the EPA Radiation Advisory Council (RAC) that was charged with reviewing BEIR VII and recommending how EPA should implement the BEIR VII report in setting radiation risk standards. For the most part, EPA accepted the BEIR VII recommendations with some modifications as recommended by the RAC. A complete review of the cell and molecular data was presented to the RAC and as part of the uncertainty analysis it was included in the recommendations to EPA. The RAC pointed out that one of the largest uncertainties associated with the risk in the low dose region was the model that was used to extrapolate the risks from high doses to the low dose region. A summary of the cell and molecular data was prepared for EPA, much of which was generated by the Program, it was included in the recommendations as an appendix. Thus, the data was considered but at the present time was not adequate to influence the setting of standards.

II. The Program's Impact on Risk

One of the first reviews of the low dose data was abook chapters published on data and results from the Program which appeared in 2006 in *Advances in Medical Physics*, edited by A.B. Wolbarst, R.Zamenhof and William R. Hendee. This chapter, "Biological Effects of Low Doses of Ionizing Radiation" represented a good balance of viewpoints and data from the molecular to

epidemiological data (Brooks 2006; Brooks et al. 2006a). Topics included an overview of BEIR VII, the role of cell-cell communication and the bystander effect which demonstrated that the responding target was much larger than the "hit" cell; a review of the adaptive protective responses and reported molecular, cellular and experimental animal data to supporting it; genomic instability and its role in radiation induced cancer; molecular changes induced by radiation in the high and low dose regions with data to support the differences in the biological mechanisms as a function of radiation dose;and an overview of "limits" of detection of biological changes indicating that in the low dose region there are thresholds or limits below which biological changes cannot be detected. This publication was a good review of major points and datasets generated by Program researchers that had an impact on setting radiation standards.

The theme of the 44th Annual meeting of the National Council on Radiation Protection and Measurements was "Low Dose and Low Dose-Rate Radiation Effects and Models." Several presentations were given on molecular, tissue and animal responses to low doses and dose-rate radiation, and this meeting resulted in several publications associated with the application of research data to standards. In most cases it is difficult to relate such studies directly to risk but these studies impact the database for determining the role of radiosensitivity (Kato et al. 2009), dose-rate and dose distribution (Brooks et al. 2009), and molecular factors that modify cancer risk (Kennedy et al. 2006; Barcellos-Hoff & Nguyen 2009; Morgan & Sowa 2009). The current and past epidemiological studies were reviewed and several studies were presented where the dose and dose-rate were very low. It was demonstrated that for some types of cancer there are non-linear dose-response relationships while when the total solid tumor data is evaluated the dose-response is linear over the whole range of doses (Gilbert 2009; Shore 2009). The meeting featured a debate and papers from Dr. David Brenner representing the BEIR VII committee and Dr. Dietrich Averbeck discussing the French Academy Report and point of views.

An important session in this meeting was "Low Dose Radiation Effects, Regulatory Policy and Impacts on the Public," in which the problems associated with incorporating mechanistic data from the Program into regulatory decision-making were discussed (Locke 2009). The weight-of-evidence approach was recommended, and interactions between scientists working at the molecular, cellular and epidemiological levels of biological organization were deemed essential for any of the information from the Program to impact standards. Each of the government agencies also made presentations about their unique problems and needs for use of mechanistic data in standard setting.

They all agreed that the standards should be based on the best scientific data available but have a wide range of different problems and reasons that this is difficult to do. Their presentations incorporated other input essential in decision making and demonstrated that science is only one element in standard setting and in many cases doesn't drive the decisions that must be made to protect the public from the potential effects of radiation (Tenforde & Brooks 2009).

In a presentation on how beliefs about radiation influence policy and decision making, (Jenkins-Smith et al. 2009) demonstrated that even though most scientists do not accept the LNT as the most scientifically sound method of regulating exposure in the low dose region, the majority of scientists and the public agree that using it is the prudent policy. The federal programs that reimburse the public for past radiation exposure represent a policy that was instituted through

Congressional action. The presentation illustrated that such programs may not be based on the best science. They make many conservative assumptions to determine who should be reimbursed to ensure that those exposed are not neglected. In many cases there is no attempt to determine a link between the dose, exposure, and disease. For example, the down-winders in Southern Utah were exposed to low doses (0.03 Gy) over a protracted time period. Using the LNT and making very conservative assumptions, of the small population exposed (about 25,000 people), only a very small number (<50) of cancers would be predicted to be induced by this exposure. To be politically correct and to correct the "wrong" of exposure from fallout it was determined that they would receive compensation if they live in selected areas in Utah and develop a cancer that is related to the types of cancers that were observed to be elevated in the Atomic Bomb survivors (Ziemer 2009).

Because ~40% of the population develops cancer and many common cancer types were included (bone, renal, leukemia other than chronic lymphocytic, and lung) there will be many of these cancers in any population. The Radiation Exposure Compensation Act (RECA) has approved payment of $50,000 to 11,815 individuals (Ziemer 2009). This illustrates that science does not drive the system but the need to err on the safe side is very critical in policy making.

Finally, a presentation was made on how to combine science and regulations for decision making following a terrorist incident involving radioactive materials. This presentation pointed out that, "It is important that an emergency response is not hampered by overly cautious guidelines or regulations. In a number of exercises the impact of disparate guidelines and training in radiological situations has highlighted the need for clear reasonable limits that maximize the benefit from an emergency response and for any cleanup after the incident" (Poston & Ford 2009). Recommendations must be very clearly defined for the first-responders so that unnecessary anxiety does not impede their ability to quickly respond to the needs associated with the disaster.

These type of presentations illustrate the importance of a good science background but also show that other factors are equally important in controlling radiation exposure.

This discussion demonstrates that to date, the Program has had limited impact on standards setting. However, it also shows that the Program has played a critical role in providing data and information on the responses in the low dose region that, with the development of better methods of using the data, will have an important impact. The program has helped understand the biological responses induced by exposure to low doses of radiation. The low dose research data has demonstrated that 1) the scientific community understands the biological responses following low dose radiation exposure, 2) that there are no surprises (risks much higher than the current standards) or data that suggest that we have underestimated the risk in the low dose range, and 3) that the use of the LNT is useful for controlling radiation exposures is conservative and provides an adequate and appropriate safety factor for risks in the low dose region. These low dose data support the huge data base that exists in the high-dose region to control the population risk from radiation induced damage.

The data produced by the Program has resulted in some major paradigm changes in radiation biology and will be very important for future activities associated with understanding and predicting the risks from low levels of radiation exposure.

Major Points: Impact on standards

A major U.S. report (NRC 2006) on the risk from low doses of radiation acknowledged the research from the Program, but did not use it in making risk estimates. The report cited the need for more mechanistic data before the findings from the Program can be applied to risk. The French Academy of Science reviewed the data on the effects of low doses of radiation including that produced by the Program and recommended that the LNT was not valid for estimating risk following low doses of ionizing radiation. They suggested that the use of LNT would overestimate the risk in the low dose region. International organizations, UNSCAR and ICRP, both issued reports which acknowledged the research from the Program. Nevertheless, both of these organizations and reports continued to use the LNT to calculate risk from the human epidemiological data. Regulatory gencies (EPA and NRC) with the responsibilities for setting radiation standards reviewed the new data from the Program, evaluated the BEIR VII and French Academy reports and accepted the more conservative recommendations for continued use of the LNT. The 44th annual meeting of the National Council for Radiation Protection and Measurements was focused on the responses in the low dose region and resulted in a good compilation of the data generated by the Program. To data the basic biology has had little impact on changing standards or regulations used to control radiation exposure. The data from the Program is widely recognized as important and continued effort is needed to insure that risk estimates and standards are based on the best scientific data available.

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Appendix A

BERAC Report Program Plan

Biological effects of Low Dose and Dose Rate Radiation

Prepared for:
Office of Biological and Environmental Research

By
BERAC Subcommittee

II. EXECUTIVE SUMMARY

There has always been natural background radiation present our environment. In addition, there is a high frequency of naturally occurring cancers that exist in all human populations. The radiation background and the large spontaneous incidence of cancer make it impossible to determine if low levels of radiation are capable of causing cancer.

Extensive research on the health effects of radiation using standard epidemiological and toxicological approaches has been used for decades to characterize the response of populations and individuals to high radiation doses, and to set exposure limits to protect both the public and the workforce. These standards were set by extrapolation of effects from high-dose studies using modeling approaches because of the inability of science to detect cancer following low doses of radiation. Thus, the historic approach has been the Linear-no-Threshold model that requires that each unit of radiation, no matter how small, will cause cancer. This model predicts effects from radiation in low-dose regions where it is not possible to demonstrate effects. Excess cancer at low doses are thus based on calculations and not on observations.

Most of the projected radiation exposures over the next 100 years will be to low doses and low dose-rates from waste clean-up and environmental isolation of materials associated with nuclear weapons and nuclear power production. The major type of radiation exposures will be low Linear Energy Transfer (LET) ionizing radiation from fission products. The DOE Program will thus concentrate on studies of low-LET exposures delivered at low total doses and dose-rates. The program will use advances in modern molecular biological and instrumentation to address the effects of very low levels of exposure to ionizing radiation. It will concentrate on understanding the relationships that exist between normal endogenous processes that deal with oxidative stress and the processes that are responsible for detection and repair of low levels of radiation induced damage. There is a single major question associated with the radiobiology of low dose exposures, “Are there adverse health effects induced by low dose and dose-rate exposure to ionizing radiation as predicted by the Linear-No-Threshold hypothesis?” To address this major question it is also important to answer:

1) Is the damage induced by ionizing radiation and the repair of that damage different from the endogenous oxidative damage and repair present during normal life processes? High levels of oxidative damage are produced and repaired daily in our bodies. Does this repair extend to oxidative damage from ionizing radiation?
2) Can endogenous repair capability prevent cancer induction following low levels of radiation exposure? Such repair could then result in a threshold of exposure below which there is no increased cancer risk.
3) Can molecular and tissue responses to radiation-induced damage prevent or reduce development of cancer? Such responses could modify the processing of damage and/or determine whether or not damaged cells are eliminated from tissue.
4) Do genetic differences exist that result in the inability of some individuals to repair radiation-induced damage?" Such genetic differences could result in sensitive individuals or sub-populations that are at increased risk for radiation-induced cancer.

The major goal of this program is to ensure that human health is being adequately and appropriately protected. It is currently costing billions of dollars to protect workers and the public from man-made radiation exposure that is lower than the natural background levels of radiation. If it is determined that there is no risk associated with these exposures, these resources could be more effectively directed toward more critical health related issues.

The funding for research in radiation biology has decreased because the Linear-no-Threshold models were conservatively protective and the scientific tools and methods available in epidemiology and toxicology were not adequate to address questions associated with cancer risk following low doses of radiation. Research to define the genome, to understand structure-function relationships and to apply molecular biology to medical problems has resulted in the development of a range of new scientific instrumentation. These instruments and methods can be modified and applied to address basic radiobiological problems. In association with the development of instrumentation, there has been an explosion of knowledge in the fields of molecular and cellular biology. For example, it is now possible to identify the genetic basis of many diseases, to clone and amplify individual genes, to grow a wide range of critical cell types associated with cancer, and to develop trangenic animal models. All these techniques help understand and modify the expression and action of many genes. With new molecular techniques and proper application of instrumentation, it will now be possible to increase our understanding of normal processes that repair oxidative and radiation-induced damage at the molecular, cellular and tissue levels, and to determine the role of low levels of radiation in changing these endogenous processes. This research program will take advantage of the modern methods and technologies to address these important national and international issues and follow the leads that are emerging from modern biology.

The overall theme of the new research program will be to understand the endogenous processes that are responsible for maintenance and repair of radiation-induced damage. If the damage and repair produced by normal oxidative endogenous processes is the same as that produced by radiation, it is possible that there are thresholds of damage that the body can handle. If the damage from ionizing radiation is different from normal oxidative damage, then its repair, and the hazard associated with it, may be unique. To understand the relationship between normal

oxidative damage and radiation- induced damage, studies will be conducted at very low doses and dose-rates and the perturbation of the normal physiological processes characterized at all levels of biological organization. Under this major theme, there will be three major research goals.

1) To determine if there are dose or energy thresholds of exposure below which there is no significant biological change or below which the damage can be effectively dealt with by normal physiological processes. If such levels exist, there should be no regulatory concern for exposures below these thresholds since there will be no increase in risk.
2) To determine how unique genetic background may alter individual sensitivity for the induction of cancer from radiation exposures and how genetic make-up influences individual and population risks.
3) To communicate the research results to policy makers, standard setters and the public so that current thinking will reflect sound science.

Research conducted under this program will help determine potential mechanisms of cancer induction by low levels of radiation and if human health is being adequately and appropriately protected from these low levels of radiation exposures.

III. INTRODUCTION

Estimates of cancer risks following exposure to ionizing radiation are based on epidemiological studies of exposed human populations, principally the Japanese A-bomb survivors. While analyses of these populations provide relatively reliable estimates of risks for high dose and high dose rate exposures it is the effects of low doses (and low dose rates) that presents the greatest health concerns for radiation workers and the general population. The risks of cancer and mutations produced by very low doses remain a critical unresolved issue, because they cannot be directly measured in exposed populations. Conceptually, we are forced to estimate risks for low-doses and for doses received as chronic protracted exposures or low dose fractionated exposures by applying various dose response models. Currently, overall estimates of low dose risks are based on empirical linear fits of the human data that are then adjusted for low-dose and dose-rate exposures. While this approach has generally been adopted by those charged with assessing radiation risks, others have argued that it is inappropriate. Specifically, this approach may greatly overestimate the cancer risks. Among those who believe current protection standards overestimate risks, many argue that a threshold for radiation-induced cancer exists. This is a critical issue because of the potential societal and economic impact of decisions upon which these estimates of risk are based. Epidemiological data by themselves are not capable of resolving the critical questions at hand; moreover, conventional experimental approaches have gone as far as they can toward addressing low dose issues.

Through recent advances in cell and molecular biology and concomitant advances in chemical and biological technology, scientists have now created an extraordinary opportunity to definitively resolve this critical low dose issue. Specific opportunities are now at hand in four interrelated areas which are key to resolving this issue: 1) characterization of radiation-induced damage to cells and tissues and its relation to endogenous damage; 2) characterization of the repair and processing of radiation-induced damage; 3) determination of the molecular and tissue

responses to radiation damage and the consequences of these responses; and 4) defining the impact of susceptible subpopulations on low dose risks.

Over the last several years it has become clear that oxidative free radicals produced by normal cellular metabolism are involved in the production of endogenous DNA damage. The types of damages produced by these free radicals overlap with the majority of molecular damage produced by ionizing radiation. Cellular DNA repair mechanisms, that are highly conserved across species, evolved to remove these endogenous oxidative DNA damages and thus preserve genomic integrity. It is precisely because free radical-induced DNA damages are efficiently repaired that cells have low rates of spontaneous mutagenesis. The question then arises as to whether low levels of ionizing radiation can be efficiently repaired by the same or similar repair systems as endogenous damage resulting in a threshold in the dose response curve. There is ample evidence that DNA repair competence can influence radiation effects, including radiation-induced cancer. There is also accumulating evidence that even low doses of radiation can elicit numerous molecular responses that have the potential to influence consequences. The above considerations support the view that a threshold at low doses of radiation may exist. With the development of sophisticated molecular biological approaches, together with new and evolving chemical and biophysical techniques, it is now be possible to readdress the low-dose issue, including the likelihood of a threshold during the next decade. Coupled with the biological program, new technologies will have to be advanced, including new approaches to measure DNA damage in the very low dose range and to determine molecular responses to such damage at the level of single genes or small changes in gene expression. Much of this technology will be facilitated by interactions with other ongoing programs such as the genome and structural biology programs.

Recent epidemiological and genetic studies suggest there may be a large number of genetic polymorphisms with the potential of conferring an increased risk for cancer as a result of interactions with environmental factors, including radiation. If the frequencies of polymorphisms that impact susceptibility to radiation-induced cancer are relatively high, it could significantly impact risk estimates at low doses for the population in general. It is now possible to identify, map, and clone the genes involved in radiation damage response functions, define the polymorphic frequencies of these genes in the population and determine their importance for susceptibility. This will provide the opportunity to directly determine their impact on cancer risk estimates after exposure to radiation.

IV. PROGRAM OUTLINE

A. THRESHOLDS

Key Question: Is there a threshold for low LET radiation-induced cancer?
The linear-no-threshold model states that cancer risk increases as a linear function of dose. From such a model it follows that even the smallest dose of radiation is theoretically capable of producing at least some cancers. It therefore becomes important to establish the validity of this model at very low doses. At issue is whether there are thresholds below which no excess cancer or genetic damage is induced. This is a difficult issue to approach experimentally because of the inability to actually measure cancers produced by very low doses. There are several types of

thresholds that have been suggested. There are statistical or practical dose thresholds below which no increase in cancer can be detected because of the severe statistical limitations imposed by the high background rate of cancer and the low frequency of radiation induced cancer. There are potential energy thresholds related to track length and structure, especially for low LET radiation, where the amount of energy deposited in a biological system is not adequate to cause produce biological damage. Finally, biological thresholds have been postulated to exist that are dependent on biological repair processes acting on radiation induced damage. The prime goals are to determine whether or not biological or energy thresholds exist following very low doses of ionizing radiation.

The existence of thresholds depends on a number of factors. It must first be determined whether the spectrum of DNA damage produced by ionizing radiation at low doses is qualitatively or quantitatively different from those produced from endogenous sources. The majority of damage produced by low LET ionizing radiation is due to the radiolysis of water in the vicinity of the DNA molecule, leading to free radical-induced DNA damages, which is similar to that produced by endogenous free radicals. However, unlike endogenous damage, production of multiple radicals close to the DNA molecule by ionizing radiation can result in highly localized clusters of damage on the DNA molecule that may be difficult to repair. For example, ionizing radiation is more efficient at producing potentially lethal DSBs. The numbers of qualitatively different lesions compared to the overall spectrum of endogenous damage must be assessed with accuracy at low doses. In addition to determining similarities and differences between the lesions initially produced by ionizing radiation and those resulting from endogenous damage, it is important to ascertain if they are inefficiently or efficiently repaired and whether the repair and processing of the radiation-induced damage results in faithful restoration of genomic integrity. Since ionizing radiation induces an stress response, in many ways similar to many endogenous oxidative processes (e.g., inflammation), it is also important to know what genes are induced in response to low dose radiation exposures and how might the induced genes influence the outcome of radiation damage to the cell.

B. NATURE OF RADITION INDUCED DAMAGE

1) **Key Question:** Is the DNA damage produced by ionizing radiation at low doses qualitatively and/or quantitatively different from endogenous damage?

2) **Description:** The majority of radiation-induced DNA damage results from free radical attack on the DNA sugars and bases, producing single strand breaks, sites of base loss (alkali-labile lesions) and a large number of modified DNA bases. A much smaller number of DSBs produced by direct ionization of DNA or possibly by the processing of multiple single lesions produced in close proximity. Protein-DNA cross-links are also formed, but in very low amounts. In spite of the fact that the frequency of DSBs is much lower than that of other types of damage, in mammalian cells, the DSB is considered to be the primary lesion involved in cellular lethality. This is because DSBs are more difficult to repair with fidelity. Clustered DNA damage that, at least at high doses, appears to be unique to ionizing radiation is particularly difficult to repair (Ward 1994). Free radical-induced lesions present on a single strand of DNA have not generally been implicated in cell death because they are readily repaired by the base excision repair system and because a correct copy of the information is present on the complementary strand. Although

the impact of unrepaired DNA damage to vital genes cannot be ignored, it is likely that subsequent processing leading to misrepaired DNA damage is largely responsible for chromosomal aberrations, genomic instability and ultimately carcinogenesis.

3) **Decision Making Value:** The problem facing scientists and policy makers is that all the information for radiation-induced DNA damage is at high doses where cells are traversed by multiple ionization tracks. There are no data at the low doses normally considered relevant to public health issues where a cell may only be traversed by a single electron track. It is not difficult to imagine that the spectrum of damage at such low doses may be substantially different from that observed at high doses. Because the background of spontaneous damage produced by free radicals derived from oxidative metabolism appears to be fairly high (Wallace 1997), the question arises as to whether low levels of ionizing radiation significantly add to the background level of damage. Thus it is fundamental to the entire low dose issue to determine whether the amount and kinds of DNA damage produced at low doses are different from those produced endogenously. If the DNA damage produced by low doses of ionizing radiation, is qualitatively similar to the damage produced by normal physiological processes then the amount of damage from the radiation is so small relative to the normal damage that it cannot have an impact on cancer risk. This would support a threshold for radiation-induced cancer. On the other hand, if ionizing radiation produces unique types of DNA and cytogenetic damage that are not produced by normal endogenous processes, the linear-no-threshold model may be supported.

4) **Recommendations and Costs:** *Characterize and quantify the spectrum of radiation-induced damage at low doses and its relation to endogenous damage.*

Characterizing and quantifying damage after very low radiation doses and placing it in context of endogenous damage is critical to this program and will require a major effort. For this effort to be successful, a significant investment in technology development will be required to expand capabilities for identifying and quantifying such damage beyond those currently available. Methodologies having high sensitivity as well as high signal-to-noise ratio will be critical in this effort. Coupling laboratories involved in characterization and quantification with groups with expertise in technology development will facilitate progress in both areas simultaneously. Once these new methods are in common use, the ten-year goal for determining the relationships between endogenous and radiation-induced damage and its repair should be realized. The initial investment in technology development will have to be in the order of $ (DOE staff), while the research programs to answer the critical questions should cost $ (DOE staff).

C. DNA REPAIR AND PROCESSING

1) **Key Question**: Does efficient repair and processing or radiation-induced damage at low doses create a threshold for radiation-induced cancer.

2) **Description:** In mammalian cells, the principal DNA repair pathways that are involved in the repair of damage to DNA resulting from ionizing radiation are base excision repair and non-homologous end-rejoining. Base excision repair, which evolved to protect cells against endogenous genotoxic damage, removes all the radiation-induced single DNA lesions, base damages, single strand breaks and sites of base loss which together account for about 70% of the

radiation-induced DNA damage (for a recent review see Wallace, 1997). This is a simple DNA repair pathway that is well understood and is highly homologous between bacteria and humans with many of the proteins exhibiting up to 40% identity. This pathway is relatively error free in most instances. Interestingly, a confounder specific to ionizing radiation is that multiple single lesions formed in close proximity to one another are recognized by the enzymes of the base excision repair pathway and their processing results in a DSB.

Double strand breaks in mammalian cells are generally repaired by non-homologous end-rejoining. This type of repair does not require homology between the two recombining molecules and is distinct from homologous recombination. Although less well characterized than excision repair, this pathway is extremely important with respect to radiation effects. This is because radiation-induced DSBs, while lower in frequency than most other types of radiation-induced damage, are the major threat to genomic integrity because of the problems associated with their repair. Mammalian cells and mice defective in components of this pathway are hypersensitive to the cytotoxic effects of ionizing radiation. Recent studies of cancer prone human populations have served to underscore the potential importance of this pathway. Cells deficient in ATM (the recently cloned gene associated with the disease Ataxia Telangiectasia) are defective in damage checkpoint controls, are sensitive to ionizing radiation, and have increased levels of spontaneous and radiation-induced chromosome aberrations. More recently, it has been demonstrated that another protein complex associated with non-homologous end-rejoining is defective in patients with Nijmegen breakage syndrome (Carney et al., 1998, Varon et al., 1998). Like patients with Ataxia Telangiectasia, individuals with Nijmegen breakage syndrome are cancer prone, radiation sensitive and demonstrate increased levels of chromosomal instability. Interestingly, the BRCA1 and 2 genes, found to be defective in many patients predisposed to breast and ovarian cancer, also appear to be involved in DSB repair pathways.

Because of the nature of the damage the non-homologous end-rejoining pathway is more error prone. Subsequent processing lead to mutagenesis, chromosomal aberrations and perhaps genomic instability. These consequences can also reveal important information relevant to the low dose question. For example, newer chromosome painting techniques have revealed that an unexpectedly large proportion of radiation-induced chromosome aberrations is due to exchanges requiring multiple breaks and involving multiple chromosomes (Savage and Simpson 1994). By earlier techniques such rearrangements appeared to be simple exchange events between chromosomes. These newer results present a clear challenge to current theories including key aspects that underpin the linear no threshold dose response.

A further challenge to current paradigms comes from recent observations on radiation-induced genomic instability. It has now been clearly demonstrated that radiation can induce changes in cells that result in an increase in mutations, chromatid type aberrations, chromosome translocations, and a decrease in cloning efficiency in the progeny of irradiated cells many population doublings after irradiation. The induction of genomic instability is postulated to be the underlying event that leads to the cascade of genetic changes that results in the genetic diversity observed in most solid cancers. What may appear to be unique about radiation-induced instability is its high frequency and makes a strong argument that it is not produced as the result of a change in a single gene or even a group of genes. Since the target for induction of genomic instability is located in the cell nucleus (Kaplan and Morgan 1998) the high frequency suggests

the target size is likely to encompass a large fraction of the genome. Genomic instability has been demonstrated in both *in vitro* systems (Kadhim et al. 1992) and *in vivo* using mice (Ponnaiya et al. 1997).

3) **Decision Making Value:** The repair of radiation-induced DNA damage is of fundamental importance to all aspects of a cell and/or an organism's responses to radiation exposure. The fidelity of the repair and damage processing systems will significantly affect the dose response curve for cancer induction, particularly at low doses. Ineffective repair or misrepair of radiation damage and subsequent processing of this unrepaired or misrepaired damage can significantly impact genomic integrity resulting in radiation-induced mutagenesis, chromosomal aberrations, chromosomal stability, and cancer. Quite simply, if radiation-induced damage is faithfully repair and processed, a threshold is expected. On the other hand, if repair and subsequent processing can lead to errors at low doses but not at high doses, an expectation of a threshold is not warranted. Additional understanding of the molecular mechanisms involved and in the closely linked damage signaling pathways will provide information relevant to the faithful repair of specific lesions, the molecular responses of cells to specific lesions and the consequences of cellular processing of radiation-induced damage compared to that of endogenous damage. Many of these consequences can be assessed using rapidly developing molecular cytogenetic technology such as combinatorial FISH. Because cytogenetic effects represent the synthesis of damage induction, repair and processing, these new technologies provide the opportunity to directly test certain key predictions of models of radiation effects at low doses.

Observations over the past few years, demonstrating the delayed radiation-induced genomic instability, are not readily understood using current radiobiological principles and paradigms. These observations have obvious important implications in understanding radiation effects in general and mutagenesis and carcinogenesis in particular. Developing a mechanistic linkage between cellular responses to low doses of ionizing radiation, genomic instability, and cancer risk or susceptibility is an important part of this program. The study of radiation-induced genomic instability provides the opportunity to: 1) identify cellular target(s); 2) clarify the role of DNA, cellular and tissue repair and the role of cell killing, proliferation and apoptosis on the induction of instability and the development of mutator phenotype; and 3) provide a framework for understanding risks following exposure to very low doses and dose-rates.

The mechanistic understanding derived relative to the repair processes at the tissue, cellular and molecular level can potentially impact current radiation paradigms and policy. The effective removal of damaged cells from a population through repair can result in biological and energy thresholds which need to be defined as a function of dose. The existence of such thresholds could modify clean-up goals and help address the question of "how clean is clean enough?" Such thresholds may also impact setting radiation exposure levels for appropriate health protection. If there are damaged cells that escape this repair process, even after very low doses of radiation, the linear-no-threshold hypothesis and current radiation paradigms would be supported.

4) **Recommendations and Costs:** *Determine the biological significance of simple base damage compared to DNA lesions of higher complexity.*
Considering the numbers involved, base damage might be considered the most important

pathway in repairing DNA damage. However, virtually all of this damage is repaired efficiently and that the vast majority of such damage is similar to damage produce by reactive oxygen species generated through normal cellular processes. The increase in DNA damage from reactive oxygen species (ROS) produced by low doses of radiation is insignificant compared to endogenous damage produced by ROS associated with normal cellular function. Therefore, research relevant to low dose effects should concentrate on damage that is unique to radiation. If specific lesions can be identified that are of particular significance for subsequent biological effects, by knowing their dependence on energy deposition and patterns of deposition, it should be possible to predict the likely form for the dose response for their production with considerable reliability.

Identify the pathways involved in damage signaling and processing of damage at low doses of radiation and the biological consequences.
The processing of initial damage to DNA often leads to misrepair products that are complex in nature, involving more than simple end-rejoining reactions. Examples include chromosomal exchanges that involve several chromosomes as part of the same event. At the molecular level there is evidence that otherwise simple exchanges involve co-deletions where large fragments of DNA are lost. Substantially more information is need on 1) the underlying repair processes; 2) sequence context and chromatin structure that may conceivably affect radiation response and target size for biological endpoints relevant to cancer; 3) how such processing leads to mutagenesis, chromosomal aberrations, and genomic instability.

Determine the mechanisms and significance of radiation-induced genomic instability for cancer risk.
Current evidence suggests that DNA repair and processing of radiation damage can lead to instability in the progeny of irradiated cells and that susceptibility to instability is under genetic control. However, there is virtually no information on the underlying mechanisms and how the processing of damage leads to instability in the progeny of irradiated cells several generations later. Further, while there has been considerable speculation about the role of such instability in radiation-induced cancer, its role in this process remains to be determined.

The technical ability to measure specific lesions and to create such lesions in genetic material, thereby facilitating studies of their significance has been a limiting factor. However many of these problems appear to be solvable and are likely to be overcome in the next few years with appropriate incentive from a program such as this for development. Progress in understanding the mechanisms of DNA repair, the interactions between repair complexes, and the structure of repair enzymes is progressing at an amazing rate. Understanding radiation-critical target interactions and subsequent DNA repair after low dose, low-dose rate radiation exposure will be facilitated by close interactions with the genome project and structural biology programs. As a result, it is highly likely that the questions outlined above can be addressed within a 10-year time frame at a cost of (DOE staff).

D. BIOLOGICAL RESPONSES AND CANCER

1) **Key Question**: Do the molecular responses induced by low doses of radiation protect cells against radiation damage or radiation-induced cancer?

2) **Description:** Damage signaling and response pathways are key elements in damage repair and processing, cell-cell interactions and cellular microenvironment. While there has been a significant amount of research defining radiation-induced genes and radiation-induced stress responses in mammalian cells, the relative contribution of a particular inductive response to the cellular consequences (survival, apoptosis, transformation) has been examined in detail for only a few genes (such as p53 or PKC). At low doses no relationship between radiation-induced responses and other oxidative stresses have been defined. Most radiation-induced gene changes reported to date are transient events, occurring at a specific time following exposure and then decreasing some time thereafter. The kinetics of these responses appear to vary with radiation dose, radiation quality, and cell type but systematic studies on specific radiation-induced responses have not been carried out. It must be determined which proteins are specifically induced in response to low doses of ionizing radiation, how these relate to other oxidative stresses, and importantly, how the induced proteins affect endpoints relevant to radiation-induced cancer. There is already some evidence that molecular, cell and tissue responses can influence radiation effects. This evidence has served to challenge current radiobiological theory that underpins the linear no threshold model. Over the last decade, a number of studies have demonstrated an apparent adaptive response in cells irradiated with small doses of ionizing radiation which manifests itself as an increased resistance to the induction of radiation effects of subsequent higher doses of ionizing radiation (Wolff 1998). Although the initial endpoint was chromosome aberrations, adaptive responses to mutation, cytotoxicity, and neoplastic transformation have been observed in vitro and in mice, induction of resistance to life shortening and the induction of thymic lymphoma have been found. It is likely that radiation-induced adaptation involves DNA repair, signal transduction and/or cell cycle kinetics. Most evidence indicate the adaptive response is related to oxidative stress and is associated with excision repair, although restriction enzymes that produce DSBs have also been shown to induce the adaptive response to ionizing radiation. More recently, several laboratories have demonstrated changes in gene expression (Le et al. 1998) , increases in sister chromatid exchanges (Nagasawa and Little 1992) and induction of cytogenetic instability (Kadhim et al. 1992) in cells not directly irradiated but rather in proximity to irradiated cells. Biological changes in cells not traversed by radiation have been called “bystander” effects. The mechanisms involved to induce bystander effects are under investigation and will help understand the mode of action of radiation. To date, bystander effects are limited to high LET radiation. It is important for this program to determine if these effects can be induced by exposure to low LET radiation delivered at low total doses or dose-rates.

3) **Decision Making Value:** An essential component of the low dose program is the determination of the functional significance of gene/protein inductive processes at low doses, and the impact on the damage response and processing pathways. Such responses could influence not only cellular responses to radiation damage but also cell-cell interactions and the interaction of cells with their microenvironment (Bissell 1998) by modifying radiation-induced damage and impacting the target size for radiation-induced cancer. Because they represent a clear challenge to radiobiological theory upon which the linear no threshold model is based, understanding the underlying mechanisms of the adaptive response and bystander effects is likely to provide important insights into critical pathways which directly impact cancer risks. All of these outcomes have important implications for effects at low doses and would argue against a

linear extrapolation from high to low doses. Rather, a significant role for inductive processes would provide a further basis for the consideration of a threshold. To properly evaluate the potential impact of such responses for cancer risks, it is essential to focus on cells and tissues that are targets tissues for radiation carcinogenesis and on low dose and low-dose rate effects.

4) **Recommendations and Costs:** We recommend that research be focused on end-points that are important in cancer formation. A major thrust of this research will thus be directed toward defining such endpoints. This will require that all questions be addressed both in appropriate cell and tissue cultures and in carefully selected experimental animal systems. This *in vitro/in vivo* approach will help insure that radiation induced changes in isolated cell systems are relevant to carcinogenesis.

These questions require a multidisciplinary approach to characterize responses at low doses and to link these responses to cancer risk. This will require better knowledge of key endogenous processes, the influence of low radiation doses on these processes, and the impact of the interaction between these processes in endpoints of direct significance for cancer risk. Measurement and characterization of these responses will require the development and application of new instrumentation and analytic technology. The overall cost of this program will be (DOE staff).

Endogenous Factors
Studies that help define the normal endogenous processes and how low doses of radiation modify these processes are recommended. This will be done by using state of the art instrumentation combined with modern cellular and molecular biological tools to link cellular and molecular changes with radiation-induced cancer.

Dose-Response Relationships
Exposure- and dose- response studies should be conducted to determine if the basic mechanisms of radiation action following exposure to low-LET ionizing radiation change as a function of total radiation dose and dose rate. High doses of ionizing radiation induce matrix and tissue disorganization, cell killing, changes in cell proliferation kinetics, induction of a multitude of genes and growth factors, and extensive chromosome and genetic damage. It is important to determine the dose of low-LET radiation that can induce these biological changes. It will also be important to determine if cancer can be induced by doses that are too low to produce such changes.

Link Low Dose Biological Response Endpoint with Cancer
Recent research has detected a number of unique cellular and molecular changes following exposure to radiation levels where biological changes had not been detected in the past. Changes such as genetic instability, adaptive responses, induction of radiation repair genes, changes in expression of stress related genes, and bystander effects all are detected following low doses of ionizing radiation. Studies to understand the mechanisms involved in these biological changes and their role in cancer induction are required. Such studies may impact many current paradigms associated with the carcinogenic mechanisms of radiation action.

Methods and Instrumentation Development

Application and development of improved instrumentation as well as advances in cellular and molecular techniques are needed to detection unique radiation induced biological changes. Research in this area is recommended. After detection of biological changes induced by low doses of radiation it again becomes essential to conduct studies to link the cellular and molecular changes observed to the induction of cancer *in vivo*.

E. GENETIC SENSITIVITY AND RISK

1) **Key Question:** Is there a distribution of sensitivities of the human population to the carcinogenic effects of radiation? How does this distribution influence risk estimates?

2) **Description:** During the last decade there has been a progressive increase in understanding of the genetic contribution to complex diseases including cancer. Molecular studies examining the genetic component of cancer susceptibility have lead to the identification of a number of genes conferring susceptibility and the number of such genes is continuing to increase. It seems likely that there may also be individual differences in susceptibility to radiation-induced cancer, and recent developments have suggested a mechanistic linkage between cellular responses to ionizing radiation, and cancer susceptibility. Though phenomenologically based, dose response kinetics for the induction of certain types of cytogenetic damage have been shown to correlate with cancer susceptibility. There is clear evidence in mice and humans for genetic control of susceptibility to radiation-induced genomic instability that may extend to cancer susceptibility as well. Further, physical associations between gene products involved in the response and repair of DNA damage as heteropolymer complexes and their apparent disruption in heritable diseases associated with instability and cancer have been recently described (Carney et al., 1998; Patel et al., 1998). Functional associations linking cell cycle, apoptosis and DSB repair have also begun to be defined in detail which offer further possible pathways for cancer susceptibility (Woo et al., 1998). However, except for rare genetic conditions affecting single genes of high penetrance, there is insufficient information to identify such potential susceptibility genes, estimate the frequency of polymorphisms in these genes in the population, and assess the risks they impose. Molecular technologies provide powerful new ways to analyze the mammalian genome and address these issues. As this area of research matures, more complex issues of genetic interactions, including gene modifiers and gene-gene interactions will be able to be addressed.

3) **Decision Making Value:** Studies focusing on genetic susceptibility to radiation-induced cancer will improve the understanding of low dose risks, but will also create opportunities for new basic knowledge of potential wide-ranging importance. Such insights will provide a better understanding not only of the basic process of cancer development but also a clearer appreciation of the interactions of both endogenous and exogenous risk factors. This is an area for which little information is available. The extent to which these studies are likely to impact current understanding of low dose risks depends on the frequency of susceptibility genes in the general population, and their ability to significantly influence low dose risks. If there are enough people who are unable to properly respond to and process radiation damage, then any model of radiation risk to the general population suggesting a threshold would appear to be untenable. It is also reasonable to assume that the distribution of such sensitive sub-populations could have a major effect on the response function of any low dose response model. The largest impact would result from changes in the slope of the initial response function. Such information will also create

opportunities to specifically identify susceptible individuals as well as provide insight into approaches to modify such susceptibility.

4) **Recommendations and Costs:** Determine the frequencies of polymorphisms in genes involved in repair and processing of radiation damage.

The efforts to identify polymorphisms in genes involved in radiation susceptibility and determine their frequencies in the population would strongly complement new initiatives at NIH and NIEHS focusing on genetic susceptibility. The interests of the low dose program effort in DOE will be unique in that the focus is on radiation damage response pathways which means that genes in the less well-characterized end-rejoining pathways will be a major interest. Because many such genes are only now being characterized and many of the known genes are quite large, these pathways have not been given high priority by other programs interested in genetic susceptibility. This aspect of the program will be closely tied and rely heavily on ongoing results and technical developments in the genome project. Like the genome and structural biology efforts this low dose effort should be coordinated with activities in the other agencies to prevent duplicative effort and facilitate rapid progress. Frequencies of identified polymorphisms will require genetic epidemiological analyses based on principle derived from population genetics. The total cost of this part of the effort is approximately (DOE staff)

Determine the biological significance of polymorphisms with respect to cancer risk. Identification of polymorphisms is only the first (and perhaps the easiest) step in the program to examine genetic susceptibility. The determination of biological significance is the ultimate goal and the more difficult task. The genome project and structural biology program each play an important role in providing guidance on which polymorphisms are most likely to influence gene function. Population genetics and computation biology approaches that are also integral to the genome project will be required to estimate the potential impact on population risk estimates. Genetic epidemiology approaches also would be of value to relate specific polymorphisms and combinations of polymorphisms with cancer risk. Another potential resource for identifying significant polymorphisms is the inbred mouse strains which have well-characterized broad differences in susceptibility to radiation-induced cancer. Direct assessment of the biological significance of candidate “susceptibility genes” can be undertaken using animal models such as knockout and knockin mice. Other animal models which emerge as part of the genome project (e.g., drosophila models) should also be considered since they may allow experiments to be performed more rapidly than with murine systems. The total cost of this aspect of the low dose program is estimated to be (DOE staff)

F. RISK COMMUNICATION

1) **Key Question:** How can the information derived from the low-dose initiative be best communicated to scientists, policy makers, stakeholders and to the public?

2) **Description:** The low-dose research program is expected to produce important new scientific data that may modify existing paradigms associated with radiation induced health risk. Since a new risk paradigm has the potential to impact existing standards and methods used in management of low-dose radiation exposures, communication between the scientific community,

policy makers and the public about the potential risk associated with radiation induced disease is vital to the outcome of the low-dose program.

Communicating the results of this research program will be a difficult challenge, since simply presenting scientific findings will not automatically impact risk policy or increase public understanding and acceptance. Influencing policy decisions will require a major change in philosophy by stakeholders and policy makers. For this shift to occur, they must have a good understanding not only of the underlying science and its implications, but also confidence that the public will accept any changes. It is well established that the public is extremely sensitive and adverse to the issue of radiation exposure (Slovic 1996). A high percentage of the public believes any exposure to radiation is likely to lead to cancer. The linear-no-threshold hypothesis supports this public conception and fosters the view that no expense is too great to reduce the risks of radiation exposure or environmental contamination. Therefore, it is not surprising to find that radiation controls tend to be associated with extremely high costs per year of life saved (Slovic 1987; Tengs, et al. 1993).

3) **Decision Making Value:** The information derived from the DOE Low Dose program must provide input for decision making but also for public acceptance of risk policy. For the decision making process, it is essential that there is adequate communication between the scientists involved in generation of the primary data and between scientists and those involved in risk policy and risk communication. Through this program the policy makers should have timely understandable scientific information which enables them to make good decisions and communicate these decisions to the public. This communication must not be one way, opportunities for public input to the decision making process is essential.

Effective communication of the results from this program should also foster better public understanding of low dose radiation risk. Communication between the scientific community, the policy makers and the public about the potential risks associated with radiation induced disease is vital to the outcome of the DOE low dose program. Good communication will solve problems regarding low dose radiation, facilitate the best policy choices and develop public understanding and support.

4) **Recommendations and Costs:** Develop a public communication program based on principles developed through risk communication research.

To communicate with the public about low dose management, requires a well-developed plan based on strong basic social science research. The goal of research effort would be to understand the likely public responses to scientific findings from the Low Dose Program’s research and responses to the plans for modifying existing standards based upon these scientific findings. The following topics should be included in determining public responses to issues regarding low dose exposures: 1) public perceptions of risk; 2) the perceived importance of the activities and conditions that produce low dose radiation; 3) trust and confidence in risk managers, regulators, and decision makers; 4) the role of the media in characterizing different positions on risk controversies; 5) the role of advocacy groups; 6) the manner by which risk is characterized and assessed; and 7) the procedures by which decisions are made. The cost for this research effort is (DOE staff).

Develop a public education program based on principles derived from risk communication science: To present the developments from this program in a form that is useful and easily understood by the public, the education program would develop web pages, written resources for public schools, and coordinate multimedia coverage of research results and public meetings. The public meetings would provide opportunities for the public to meet with scientists and regulators involved in policy making, facilitating public input into the decision making process. The cost of this public education program will be (DOE staff).

Develop a communication network between scientists, policy makers, and DOE administrators: The low dose program is highly dependent on effective interactions and collaborations among scientists with varied scientific and technical expertise. For this to be successful, a communication network must be developed which will ensure adequate communication. This network would encompass not only the scientists directly involved in the conduct of studies as a part of this program but also those involved in the genome and structural biology programs. An expanded network including scientists, policy makers from a variety of agencies, and DOE administrators is required to keep the program focused on critical issues and facilitate the understanding and translation of result into public policy. The costs for development and maintenance of this active network will be (DOE staff).

V. PROGRAMMATIC STRUCTURE, MONITORING PROGRESS, DIRECTION AND FOCUS

The research themes identified in this program are to address the questions that provide information that meet the DOE needs for improved understanding of radiation risks at low doses. The questions that can impact current radiation paradigms are as follows: 1) Is radiation induced DNA damage different than endogenous oxidative damage? 2) Can normal physiological processes repair the DNA damage and prevent cancer following low levels of radiation exposure? 3) Can organ, tissue and molecular responses to low doses of radiation prevent or reduce cancer risk? 4) How does genetic differences impact radiation induced cancer risk.

To achieve these goals, the proposed research program should, of necessity, be more focused than the NIH R01 model. For example, a systematic analysis of a single link in the chain of arguments that leads to the assumption of dose linearity or a threshold would be a highly appropriate task within the current context. This is not to say that scientific merit should not be reviewed as rigorously. Clearly, rigorous peer review is essential. However, research proposals for this program should explicitly reflect the framework set out here.

A critical component of this research program will be its ability to continue addressing both the original and changing goals over time. As with any basic research program, especially one that is focused on a specific challenge, program needs will change as results are accumulated from this and other research programs. In addition, as interactions between scientists in this program and at regulatory agencies develop and mature (see next section), program goals will be further clarified and new goals will be identified.

Scientific progress, at the individual project level, will be monitored and evaluated through the use of ad hoc peer review panels and occasional ad hoc mail reviews, under the guidance of BER program managers. The results of these peer reviews will be evaluated and used by BER management to make decisions on the funding of individual projects across the program. BER program managers will also evaluate progress among groups of related projects and across the entire program.

A standing BERAC low dose effects subcommittee through interactions with BER program managers will evaluate overall program progress, direction, and focus. This subcommittee should be comprised of scientists with expertise representing the entire range of program goals. In addition, the subcommittee should include individuals with expertise in or responsibility for developing human exposure regulatory policy. This committee should meet with BER program managers to assess the portfolio of grants within this program, and to recommend changes in emphasis and balance. In addition, the committee would also be charged with defining programmatic areas that require increased / decreased emphasis based results of this program and advances in other fields of relevance to this program (i.e., scientific issues), and new issues related to risk management (regulatory issues). Such recommendations may be reflected in the issue of RFP's for this program if warranted and sufficient research funds are available. The subcommittee will also participate in the low dose effects program contractor workshops (see next section) to be held approximately every 18 months. A major review of the program should be scheduled at the end of five years.

In this subcommittee, findings will be reported, in writing, to BERAC for further discussion, comment, and approval. Final reports will be distributed to scientists in the low dose effects program, BER management, the Director of the Office of Energy Research, program staff at other agencies and interested congressional staff. The reports will be publicly available in hard copy or on the BERAC web site at http://www.er.doe.gov/production/ober/herac.html. The reports will also serve as the basis for future program solicitations and for the development of special research workshops or symposia to help clarify or debate specific program topics or to inform scientists and the public on program progress and future directions.

VI. *Program Contractor Workshops – Involving Customers and Stakeholders*

The ultimate success of this program will depend on the quality of the science produced and the usefulness of that science to the people and organizations charged with using research results to develop public health protection policy. To facilitate the kinds of interactions that will improve both the science and, hopefully, the usefulness of the results for developing public health protection policy, program contractor workshops will be held approximately every 18 months.

All principal investigators funded in the low dose effects research program will be expected to participate in these workshops. BER program staff, program staff at other agencies, BERAC low dose effects subcommittee members, and scientists from other DOE-funded programs whose research has useful links to the low dose program will also be invited to participate. Finally, staff from regulatory agencies, e.g., the environmental Protection Agency, the Nuclear Regulatory Commission, etc., will be invited to actively participate in these workshops. As well as this oversight role, it is recommended that the subcommittee act in conjunction with BER program

managers to act as the Scientific Program Committee for this annual meeting. The Committee's principle charge here would be to organize a highly focused symposium on a single theme or issue, in which the current state of the art is reviewed, and potential future directions assessed.

VI. FUTURE DIRECTIONS AND RESEARCH

The goal of these workshops will be several fold. They will serve as forums for exchanging research results, for communicating and discussion ongoing or changing program directions, and as opportunities to evaluate the overall balance of the low dose research portfolio. They will serve as opportunities for scientists in the program to broaden their scientific perspectives and their understanding of how their research project fits into and contributes to the low dose effects program. Finally, and perhaps most importantly, it will provide opportunities for people involved in developing public health protection policy to discuss, with research scientists, the types of new or clarifying information that they need or can use from research.

These workshops will change the way that research scientists think about and conduct their research. They will open new lines of communication among program scientists and between those scientists and the users of the research results being developed in the program. Research results will still be published in peer-reviewed scientific journals; however, the dialogues, the exchanges of information, and the new understandings of the relationship between basic research the development of health protection policy that occur at these program workshop may be among the most significant outcomes of this research program.

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Appendix B

First Call for Proposals

The calls for proposals follow the development of the Program and the direction that was provided by DOE to maintain the Program's focus. Each call resulted in a large number of applications. After review for relevance to the Program, review groups ranked each proposal according to its scientific strengths. After this ranking, DOE applied programmatic needs, and the proposals were ranked and funded. Shown here is the text of first call, which was made in 1998.

SUMMARY: The Office of Biological and Environmental Research (BER) of the Office of Energy Research (ER)[3], U.S. Department of Energy (DOE), hereby announces its interest in receiving applications for research for support of the Cellular Biology Research Program. This Program is a coordinated multidisciplinary research effort to develop creative, innovative approaches that will provide a better scientific basis for understanding exposures and risks to humans associated with low level exposures to radiation and chemicals. Using modern molecular tools, this research will provide information that will be used to decrease the uncertainty of risk at low levels, help determine the shape of the dose-response relationships after low level exposure, and achieve acceptable levels of human health protection at the lowest possible cost.

SUPPLEMENTARY INFORMATION: Current standards for occupational and residential exposures to radiation and chemicals are based on linear, no-threshold models of risk that drive regulatory decisions and estimations of cancer risk. Linear, no-threshold models assume that risk is always proportional to dose, that there is no risk only when there is no dose, and that even a single molecule or radiation induced ionization can cause cancer or disease. However, the scientific basis for these assumptions is limited and uncertain at very low doses and dose rates.

Much scientific evidence suggests that the risks from exposure to low doses or low dose-rates of radiation and chemicals may be better described by a non-linear, dose-response relationship. This evidence includes long term human and animal studies and research at the cellular and molecular level on the DNA repair capabilities of cells and tissues, bystander' effects associated with low dose exposures, the effects of exposure-induced gene expression, the effects of a cell's micro environment on its response to low dose exposures, and studies of the multi-step nature of cancer development. A more definitive understanding of the biological responses induced by low dose, low dose-rate exposures is needed to clarify the role played by these and other cell responses and capabilities in determining risk.

This research program will focus on understanding the mechanisms of molecular and cellular responses to low dose, low dose-rate exposures to radiation and chemicals to improve the scientific underpinning for estimating risks from these exposures. The program will include

[3]Name was changed to the Office of Science in 1998.

research to identify and characterize: (1) the genes and gene products that determine and affect these cellular responses induced at low dose and dose-rates; (2) the role played by these genes and gene products in determining individual differences in susceptibility to low dose, low dose-rate exposures; and (3) methods to synthesize or model molecular level information on genes and gene products into overall health risk. The program will also communicate research results to regulators and legislators. The goal of this research program is the development of scientifically defensible tools and approaches for determining risk that are widely used, accepted, and understood.

Research is encouraged in a number of areas including, but not limited to:

- The effects of and reactions to reactive oxygen species at low doses and/or dose rates.
- The role of gene induction, DNA repair, apoptosis, and the immune system in mediating responses to low dose and/or low dose-rate exposures.
- The nature and significance of bystander' effects in determining cell and tissue responses to low dose and/or low dose-rate exposures.
- The role of cell and tissue microenvironments in determining cell and tissue responses to low dose and/or low dose-rate exposures.

Development of computational techniques, e.g., algorithms and advanced mathematical approaches, for use in determining risk, that model new information from cellular and molecular studies together with available data from epidemiologic and animal studies.

A Lead Scientist will be selected from among all investigators who are successful in receiving research funds in this program. This research program will be directed by a program manager from BER, who will be responsible for providing support and overall direction, including determining the relevance of the goals and objectives of the program. The Lead Scientist will provide scientific leadership to the community of the researchers in the research program. Applicants interested in being considered as a Lead Scientist for the low dose research program should indicate their interest in their research application. In addition to the information requested in the Application Guide, applicants should supplement their applications by describing their qualifications to serve as a Lead Scientist for this program. The supplemental information should be provided as a separate appendix not attached to the main application. Interested applicants should demonstrate their understanding of the needs for and the uses of the types of scientific information likely to be developed in this research program. They should demonstrate their understanding of previous epidemiologic and experimental studies involving low dose, low dose-rate exposures to radiation or chemicals. Finally, interested applicants should demonstrate their knowledgeability of research opportunities and capabilities at National Laboratories, universities, and industry in the area of molecular and cellular responses to low dose, low dose-rate exposures.