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Project Director: Priscilla K. Cooper, Ph.D. 2008 Program Chair
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Environmental Mutagen Society

39h Annual Meeting
Low Dose Radiation-Induced Genome and Epigenome Instability Symposium and Epigenetic Mechanisms, DNA Repair, and Chromatin Symposium
Genes and the Environment: From Molecular Mechanisms to Risk
October 18–22, 2008
Wyndham Rio Mar Resort
Rio Grande, Puerto Rico

Final Report, Agenda and Abstracts
February 19, 2009

Introductory remarks were presented for the Low Dose Symposium on a new integrated programmatic approach to studying low dose radiation effects using systems biology. The hypothesis was that radiation risk extrapolations assume that the deposition of energy by radiation is likely related linearly related to dose but the cellular/tissue responses to exposure might not be. How cells in tissues interact to modulate radiation-induced changes is poorly understood, and this is a multi-scale, systems-level challenge. This was followed by an elegant synopsis on epigenetic deregulation of targeted and non-targeted effects of ionizing radiation, summarizing work analyzing epigenetic changes in bystander and transgenerational radiation effects using animal models. The data suggested that epigenetic changes (DNA methylation histone modifications and microRNAome changes) were important in a plethora of non-targeted effects of radiation exposures. The next talk was discussed studies on how maternal exposures to low doses of ionizing radiation might alter the fetal epigenome. Evidence was presented that epigenetic dysregulation during early development is mechanistically linked to the pathogenesis of adult-onset disease. The data indicated that the protective effect of low doses of radiation may be mediated by epigenetic mechanisms that lead to increased DNA methylation at critical sites in the genome. Research on the impact of *in utero* irradiation on mutation rates at expanded simple tandem repeat DNA loci in exposed mice and their first generation offspring was discussed. Data implied that the passive erasure of epigenetic marker(s) in the maternal genome could diminish the transgenerational effects of fetal irradiation. This interesting observation implicates important clues as to the mechanisms of radiation-induced genomic instability. A presentation on investigating low dose radiation induced genomic regulation of pathways critical to altering chromatin conformation was given. New data indicated a number of previously unsuspected cellular pathways are involved in responses to low doses of ionizing radiation and may modulate specific cellular and tissue responses associated with such low dose exposures, particularly radiation induced adaptive responses. This session was particularly well attended and there was a stimulating question and answer period reflecting the burgeoning interest in effects of exposures to low doses of ionizing radiation and potential genetic and epigenetic responses.

The Epigenetic Mechanisms Symposium speakers discussed how low levels of radiation can lead to changes in the methylation status of certain gene promoters and the expression of DNA methyltransferases. However, epigenetic regulation can also involve changes in higher order chromosome structure. This Symposium focused on epigenetic mechanisms and their interplay with DNA repair and chromatin changes. It was organized by a prominent investigator in the new area of epigenetic changes in response to radiation damage, together with the 2008 EMS Program Chair. Topics covered included the role of DNA repair in promoter demethylation, chromatin decondensation at DNA double-strand breaks, the dynamic interactions between chromatin assembly factors and DNA repair, and epigenetic regulation by chromatin looping. This session was also well attended.

**TUESDAY, OCTOBER 21, 2008**

**8:30 AM – 10:30 AM** **SYMPOSIUM 13: LOW DOSE RADIATION-INDUCED GENOME AND EPIGENOME INSTABILITY**

*Chairpersons: William F. Morgan, University of Maryland, Baltimore and Matthew Coleman, Lawrence Livermore National Laboratory*

| | | |
|---|---|---|
| 8:30 AM – 8:50 AM | S70 | Low Dose Radiation Induced Genomic and Epigenomic Instability<br>Speaker: William F. Morgan, University of Maryland, Baltimore |
| 8:50 AM – 9:15 AM | S71 | Role of Epigenetic Deregulation in Targeted and Non-Targeted Radiation Responses<br>Speaker: Olga Kovalchuk, University of Lethbridge |
| 9:15 AM – 9:40 AM | 17 | Maternal Low Dose Radiation Alters the Fetal Epigenome<br>Speaker: Dana C. Dolinoy, Duke University Medical Center |
| 9:40 AM – 10:05 AM | S73 | The Effects of In Utero Irradiation on Mutation Induction and Transgenerational Instability in Mice<br>Speaker: Yuri Dubrova, University of Leicester |
| 10:05 AM – 10:30 AM | S74 | Low Dose Ionizing Radiation-Induced Genomic Regulation of Pathways Critical to Altering the Chromatin<br>Speaker: Matthew A. Coleman, Lawrence Livermore National Laboratory |

**Wednesday, October 22, 2008**

**3:15 PM – 5:20 PM** **SYMPOSIUM 16: EPIGENETIC MECHANISMS, DNA REPAIR, AND CHROMATIN**

*Chairperson: Olga Kovalchuk, University of Lethbridge*

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|---|---|---|
| 3:15 PM – 3:40 PM | S85 | Thymine DNA Glycosylase, DNA Base Excision Repair and (Epi)Genome Maintenance<br>Speaker: Primo Schär, University Basel, Switzerland |
| 3:40 PM – 4:05 PM | S86 | DNA Damage, Epigenetic Alterations and Liver Carcinogenesis<br>Speaker: Igor Pogribny, National Center for Toxicological Research, U.S. FDA |
| 4:05 PM – 4:30 PM | S87 | Poly(ADP-ribosyl)ation Causes Rapid Chromatin Decondensation at Sites of Micro-IR-Induced Double-Strand Breaks<br>Speaker: Michael Hendzel, Cross Cancer Institute and University of Alberta |
| 4:30 PM – 4:55 PM | S88 | Chromatin Assembly Factors and the Challenges of DNA Replication and Repair<br>Speaker: Genevieve Almouzni, Institut Curie, Paris |
| 4:55 PM – 5:20 PM | S89 | Genome Organizer SATB1 in DNA Repair<br>Speaker: Terumi Kohwi-Shigematsu, Lawrence Berkeley National Laboratory |

## S70

**Low Dose Radiation Induced Genomic and Epigenomic Instability.** Morgan WF. University of Maryland, Baltimore, MD, United States.

Cellular exposure to DNA damaging agents like ionizing radiation can result in direct (targeted) damage to the genetic material and a number of non-targeted indirect effects that can manifest in the progeny of a damaged cell. Both targeted and non-targeted effects can result in DNA mutations, gene amplifications, chromosomal rearrangements, carcinogenesis, and even cell death. The paradigm for understanding how induced damage results in these cellular endpoints dictates that cellular responses to the induced damage, e.g., DNA repair, and cell cycle arrest fix the damage and thereby seal the fate of the irradiated cell. However, non-targeted effects cannot be accounted for by this paradigm. This presentation will focus on delayed genetic effects occurring in the progeny of cells after exposure to ionizing radiation. We will describe how the cellular micro-environment can perpetuate instability in clonally expanded populations of cells surviving irradiation. The emphasis will be on gene expression analysis, the persistently elevated levels of reactive oxygen species and the role of mitochondrial dysfunction that characterize many of our chromosomally unstable clones. These results will be discussed in terms of non-targeted bystander like effects where by cells that themselves were not irradiated exhibit many of the same detrimental effects as irradiated cells. In addition, other non-targeted effects associated with radiation exposure including clastogenic factors, the death inducing effect, hereditary effects, and abscopal effects of radiation and how these might impact on human disease will be discussed. This work was supported by the Biological and Environmental Research Program (BER), U.S. Department of Energy.

## S71

**Role of Epigenetic Deregulation in Targeted and Non-Targeted Radiation Responses.** Kovalchuk O. University of Lethbridge, Lethbridge, AB, Canada.

While modern cancer radiation therapy has led to increased patient survival rates, the risk of radiation treatment-related complications is becoming a growing problem. Radiation poses a threat to exposed individuals and their progeny. It is known to cause genome instability that is linked to carcinogenesis. Radiation is known to cause a wide variety of indirect effects. It was proven to induce genome instability in the distant naïve out-of-field 'bystander' cells and their progeny. Enigmatically, it also affects the unexposed progeny of the pre-conceptually exposed animals and humans. Genome instability has been implicated in the latter phenomena. Yet, the mechanisms by which it arises remain obscure. We hypothesized that epigenetic alterations play leading roles in the molecular etiology of the radiation-induced genome instability. Epigenetic changes comprise cytosine DNA methylation, histone modifications and small RNA-mediated events. We analyzed the roles of aforementioned epigenetic changes in the bystander and transgenerational radiation effects using animal models. We will present new and compelling evidence that epigenetic changes (DNA methylation, histone modifications and microRNAome changes) are important for the molecular etiology of the indirect bystander and transgenerational radiation effects. Supported by the Canadian Institutes for Health Research and the Alberta Cancer Board.

## 17

**Maternal Low Dose Radiation Alters the Fetal Epigenome.** Dolinoy DC[1], Jirtle RL[2]. [1]University of Michigan School of Public Health, Ann Arbor, MI, United States, [2]Duke Univeristy Medical Center, Durham, NC, United States.

Radiation-induced bystander effect is a phenomenon where cells not directly exposed to ionizing radiation display a marked enhancement in chromosomal and genomic instability, which is thought to result in part from epigenetic changes. There is now accumulating evidence that epigenetic dysregulation during early development is also mechanistically linked to the pathogenesis of adult-onset diseases. In this study, viable yellow agouti $A^{vy}/a$ offspring were exposed *in utero* to a total dose of 8.4 cGy of low LET radiation delivered at 1.2 cGy/day from gestational day 1.5 to gestational day 8.5 using the Imtek small animal MicroCAT X-ray/CT scanner. A second set of $A^{vy}/a$ offspring was sham-irradiated *in utero* for 7 consecutive days. The results demonstrate that maternal exposure to this low dose of fractionated radiation shifts the coat color distribution of $A^{vy}/a$ offspring toward the brown pseudoagouti coat color phenotype ($p=0.01$). Quantitative DNA methylation analysis using the Sequenom EpiTYPER platform revealed increased methylation at several CpG sites upstream of the $A^{vy}$ cryptic promoter ($p<0.05$). CpG methylation was also increased at another metastable locus, the *CDK5 activator binding protein* ($Cabp^{IAP}$) ($p<0.05$). These data indicate that the protective effect frequently observed following low dose radiation exposure may be mediated by epigenetic mechanisms that lead to increased DNA methylation at critical sites in the genome. To test this intriguing postulate, additional exposure groups will be added to this study.

## S73

**The Effects of *In Utero* Irradiation on Mutation Induction and Transgenerational Instability in Mice.** Barber RC, Hardwick RH, Shanks ME, Glen CD, Mughal SK, Voutounou M, Dubrova YE. Department of Genetics, University of Leicester, Leicester, United Kingdom.

Epidemiological evidence suggests that the effects of prenatal irradiation manifest during adulthood, resulting in the increased risks of leukemia and solid cancers after birth. However, the mechanisms underlying the long-term effects of fetal irradiation remain poorly understood. This study was designed to analyze the impact of *in utero* irradiation on mutation rates at expanded simple tandem repeat (ESTR) DNA loci in the directly exposed mice and their first-generation offspring. BALB/c pregnant mice (Theiler stage 20, 12 days of gestation) were exposed to 1 Gy of acute X-rays. ESTR mutation frequencies in the germline and somatic tissues of *in utero* irradiated male and female mice remained highly elevated during adulthood, which was mainly attributed to a significant increase in the frequency of singleton mutations. The prevalence of singleton mutations in the exposed mice suggests that fetal irradiation results in genomic instability manifested *in utero* and during adulthood. To analyse the effects of parental irradiation on transgenerational instability, the frequency of ESTR mutation was established in DNA samples prepared from sperm, bone marrow and brain taken from the first-generation offspring of *in utero* irradiated male and female mice. The frequency of ESTR mutation in the first-generation offspring of prenatally irradiated male mice was equally elevated across all tissues. In contrast, the frequency of ESTR mutation in the offspring of irradiated females did not significantly differ from that in controls. Our data therefore imply that the passive erasure of epigenetic marks in the maternal genome can diminish the transgenerational effects of fetal irradiation and therefore provide important clues onto the still unknown mechanisms of radiation-induced genomic instability.

## S74

**Abstract Not Available.**

## S85

**Thymine DNA Glycosylase, DNA Base Excision Repair and (Epi)Genome Maintenance.** Schär P. Institute of Biochemistry and Genetics, Department of Biomedicine, University of Basel, Basel, Switzerland.

The human thymine DNA glycosylase (TDG) first attracted attention because of its ability to remove thymine, i.e. a normal DNA base, from G•T mispairs. This implicated a function of DNA base excision repair (BER) in the restoration of G•C base pairs following the deamination of a 5-methylcytosine (5-meC). TDG then turned out to be the founding member of a family of mismatch-directed uracil DNA glycosylases that act on a broad spectrum of base lesion, including G•U mispairs as well as lesions generated by the anticancer drug 5-Fluorouracil. 5-meC DNA glycosylase activity has also been associated with TDG, thrusting the enzyme into limelight as a possible DNA demethylase. Last but not least, TDG was found to interact with transcription factors, nuclear receptors as well as with DNA methyltransferases, implicating a function in gene regulation, which appears to be critically important in developmental processes. We have been pursuing biochemical and genetic approaches with yeast and mouse models to determine the biological function of this multifaceted DNA repair enzyme. I will present data implicating newly identified, non-redundant functions of TDG dependent BER in (epi)genome maintenance and regulation of gene expression, and discuss mechanistic aspects of cellular drug resistance and differentiation phenotypes associated with the loss of TDG.

## S86

**DNA Damage, Epigenetic Alterations and Liver Carcinogenesis.** Pogribny IP, Beland FA. National Center for Toxicological Research, Jefferson, AR, United States.

It is widely believed that DNA damage induced by various exogenous and endogenous factors is a critical event in the initiation of tumorigenesis. However, the initiation alone is not sufficient for tumor formation; rather it creates the necessary prerequisite for tumor development, which results from a much broader alteration in cellular homeostasis, particularly from the inability of cells to maintain and control accurately the normal cellular epigenomic pattern. We have conducted experiments to examine the role and contribution of DNA damage and epigenetic alterations induced by exposure to genotoxic and non-genotoxic carcinogens in rodent liver carcinogenesis. Long-term exposure of rats to the genotoxic carcinogens, tamoxifen and 2-acetylaminofluorene, and a non-genotoxic carcinogenic methyl-deficient diet resulted in accumulation of carcinogen-induced genotoxic changes, specifically in carcinogen-specific DNA adduct formation from tamoxifen and 2-acetylaminofluorene, and DNA strand-breaks from the methyl-deficient diet, and in noticeable epigenetic alterations, such as changes in the DNA methylation and histone modifications. More importantly, we found that epigenetic changes persisted after removal of the methyl-deficient diet, while the integrity of DNA was completely restored. Together, these data indicate 1) the causative role of DNA damage in disrupting the cellular epigenomic pattern; and 2) the significance of epigenetic but not genotoxic changes for tumor progression during hepatocarcinogenesis.

## S87

**Poly(ADP-ribosyl)ation Causes Rapid Chromatin Decondensation at Sites of Micro-IR-Induced Double-Strand Breaks.** McDonald D[1], Haince J-F[2], Rouleau M[2], Poirier G[2], Hendzel M[1]. [1]University of Alberta, Edmonton, AB, Canada, [2]Universite Laval, Quebec, QC, Canada.

Kruhlak and colleagues (J. Cell Biol. 172:823-834) discovered a rapid chromatin decondensation upon introduction of double-strand breaks induced by laser microirradiation. This decondensation was independent of ATM kinase activity and phosphorylation of histone H2AX. An attractive candidate for this decondensation is poly(ADP-ribosyl)ation, which we have previously shown decondenses chromatin *in vitro* (PNAS 79:3423-3427). We tested the hypothesis that this chromatin decondensation required poly(ADP-ribosyl)ation. Using multi-photon excitation to introduce double-strand breaks (DSBs) and simultaneously photoactivate PAGFP-histone H2B, we observed rapid chromatin decondensation. This chromatin decondensation was significantly reduced in cells treated with a potent inhibitor of poly(ADP-ribosyl)ation. Similariy, overexpression of the glycohydrolase, PARG, also prevented chromatin decondensation. Chromatin decondensation was significantly reduced in PARP-1 knockout mouse embryonic fibroblasts but restored upon transfection of the knockout cells with PARP-1. We conclude that poly(ADP-ribosyl)ation is necessary and sufficient for the decondensation of chromatin at sites of double-strand breaks. We have previously shown that PARP activity is required for the efficient recruitment of the MRN complex at sites of double-strand breaks thereby implicating PARP signaling as an upstream component of the DSB repair signaling pathway (JBC 283:1197-1208). MRE11 was found to contain a high affinity poly(ADP-ribose) binding domain. Thus, PARPs serve at least two important complementary roles in the repair of DSBs. It functions to “open” chromatin structure, which may facilitate the access of the DSB repair machinery to sites of DSBs. By rapidly introducing PAR at sites of DSBs, PARPs also function by concentrating DSB repair machinery at sites of DSBs.

## S88

**Chromatin Assembly Factors and the Challenges of DNA Replication and Repair.** Ray-Gallet D, Polo S, Quivy J-P, Groth A, Roche D, Almouzni G. UMR218 CNRS/Institut Curie, Paris, France.

Inheritance and maintenance of the DNA sequence and its organization into chromatin are central for eukaryotic life. To orchestrate DNA-replication and –repair processes in the context of chromatin is a challenge. Factors have been isolated from cell extracts that stimulate early steps in chromatin assembly *in vitro*. One such factor, chromatin assembly factor-1 (CAF-1), facilitates nucleosome formation coupled to DNA synthesis. It is thought to participate in a marking system at the crossroads of DNA replication and repair to monitor genome integrity and to define particular epigenetic states. We have now identified a chromatin assembly pathway independent of DNA synthesis involving the HIRA protein. Notably, CAF-1 is part of the histone H3 complex, H3.1 complex (replicative form) and HIRA of the H3.3 complex (replacement form) (Tagami et al, 2004, Nakatani et al, 2004). In addition, another histone chaperone, Asf1, has to be integrated in a network of interactions leading to nucleosome deposition. A major goal in our laboratory is now to better integrate the function of these factors *in vivo* during development and also in connection with replication, repair and control of histone pools. We will discuss our recent findings on these topics and the interrelationships with other assembly factors. Groth A. et al. (2005) *Mol Cell*, 17, 301-311. Polo S. & Almouzni G. (2006) *Curr Op Genet Dev*, 16, 104-111. Polo S. et al. (2006) *Cell*, 127, 481-493. Loyola A. et al. (2006) *Mol Cell*, 24, 309-316. Groth A. et al. (2007) *Cell*, 128, 721-733. Groth A. et al. (2008) *Science*, 318, 1928-1931.

## S89

**Genome Organizer SATB1 in DNA Repair.** Ayers S, Li J, Han H-J, Cai S, Kohwi-Shigematsu T. Life Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA, United States.

Research in the past decade has revealed the important contribution of histone modifications and nucleosome remodeling to chromatin structure and function, especially gene regulation. It still remains largely unknown how chromatin is organized into higher-order structure, and how such organization plays a role in gene regulation, and potentially in other nuclear functions such as replication and repair. We study a protein called SATB1, expressed in a cell type-specific manner, which functions in organizing higher-order chromatin folding. SATB1 is a novel global gene regulator, regulating expression of hundreds of genes by tethering its target gene loci onto its cage-like nuclear distribution and recruiting chromatin remodeling/modifying enzymes to these target genes to establish specific histone modification status at these loci. We found SATB1 knockout thymocytes are hypersensitive to ionizing radiation-induced DNA damage due to defects in the DNA repair process. I will describe the new role of SATB1 in DNA repair.