ALBERTA GIAC 2011

4th Alberta Genomic Instability & Aging Conference

May 16-18 2011

University of Calgary

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PROGRAM AND ABSTRACT BOOK

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ALBERTA GENOMIC INSTABILITY AND AGING CONFERENCE 2011

May 16 – 18, 2011

The University of Calgary
Health Sciences Centre, Faculty of Medicine

Organizers: Drs. Jennifer Cobb and Susan P. Lees-Miller

Monday, May 16 – Theatre Three

8:15 AM Registration Desk opens – HRIC Atrium

Morning Session

9:00 am Welcome and opening remarks – Jennifer Cobb

9:15 am Brendan Price (Dana-Farber Cancer Institute)
Chromatin dynamics and the epigenetics of DNA repair

9:55 am Shelagh Campbell (U of Alberta)
Wee1 regulation of the S/M checkpoint

10:25 am Coffee break

10:45 am Laura Fick (U of Calgary)
Does telomere length control the lifespan of dog breeds?

11:15 am Ismail Hassan Ismail (U of Alberta)
Pc2 regulates BMI-1 recruitment at sites of DNA damage

11:45 am Chris Williamson (U of Calgary)
Combined loss of ATM and p53 increases toxicity of PARP inhibitors in Mantle Cell Lymphoma

12:15 pm Lunch – HRIC Atrium

Monday, May 16 – Theatre Three

Afternoon Session

1:30 pm David Cortez (Vanderbilt University)
Analysis of protein dynamics at active, stalled and collapsed replication forks

2:10 pm Damien D'Amours (U of Montreal)
Decomplexifying the Smc5-6 complex: From DNA-binding activity to anti-recombination functions

2:40 pm Jennifer Cobb (U of Calgary)
The Smc5/6 complex component Nse5 regulates sumoylation and replication fork stability

3:10 pm Coffee break

3:30 pm Linda Reha-Krantz (U of Alberta)
Srs2: Guardian of lagging strand DNA replication

4:00 pm Karlene Cimprich (Stanford University)
Mechanisms for maintaining genome stability at the replication fork

Evening

5:00 pm Poster Session – HRIC Atrium

7:00 pm Banquet Dinner – HRIC Atrium

Tuesday, May 17 – Theatre Three

Morning Session

9:00 am	Andre Nussenzweig (National Cancer Insitute – NIH) Linking nuclear DNA damage and epigenetic changes to stem-cell decline during aging
9:40 am	Gordon Chan (U of Alberta) Regulation of the mitotic checkpoint – RZZ complex
10:10 am	Coffee break
10:30 am	Roger Greenberg (U of Pennsylvania) Ubiquitin recognition and remodeling at DNA double strand breaks
11:10 am	Gernot Neumayer (U of Calgary) The spindle assembly factor TPX2 regulates DNA damage response via MDC1 / ATM-dependent amplification of p-H2AX
11:40 am	Aaron Goodarzi (U of Calgary) KAP-1 phosphorylation regulates heterochromatic CHD3 nucleosome remodeling during the DNA double strand break response
12:10 pm	Lunch – HRIC Atrium

Tuesday, May 17 – Theatre Three

Afternoon Session

1:30 pm Corey Nislow (Donnelly Centre, U of Toronto)
Insights into the mechanism of action of nonclassical platinum–acridine anticancer agents from chemogenomic screening

2:10 pm Bernard Duncker (U of Waterloo)
Characterization of Dbf4 interactions with DNA replication and checkpoint factors

2:40 pm Nicolas Coquelle (U of Alberta)
Mechanistic insights on DNA recognition of PNKP phosphatase domain

3:10 pm Coffee break

3:30 pm Grant Brown (Donnelly Centre, U of Toronto)
A genome-wide view of protein dynamics during replication stress in *S. cerevisiae*

4:00 pm Marta Davidson (U of Calgary)
DNA replication stress results in expansion of dNTP pools and a mutator phenotype

4:20 pm Mireille Tittel-Elmer (U of Calgary)
The MRX complex regulates cohesin during replication: In times of stress keep your sister close

4:40 pm Michael Schultz (U of Alberta)
Functional interdependence of lysine acetylase Rtt109 and histone chaperone Asf1 in transcriptional regulation

Wednesday, May 18 – Theatre Three

Morning Session

9:00 am Daniel Durocher (Samuel Lunenfeld Research Institute, U of Toronto)
Ubiquitin signalling orchestrates the response to DNA double-strand breaks

9:40 am Haico van Attikum (Leiden University Medical Center, Netherlands)
Novel chromatin remodeling factors that promote signaling and repair of DNA damage

10:20 am Coffee break

10:40 am Michael Hendzel (U of Alberta)
Polycomb group proteins in the DNA damage response

11:10 am Carrie Shemanko (U of Calgary)
Prolactin induces breast cancer cell resistance to DNA damaging agents via a heat shock protein-90-mediated mechanism

11:40 am Susan Lees-Miller (U of Calgary)
Structural insights into the mechanism of nonhomologous end-joining

12:10 pm Closing remarks

12:20 pm Box Lunch

ABSTRACTS

ORAL PRESENTATIONS

Chromatin dynamics and the epigenetics of DNA repair

Brendan D. Price

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The compact conformation of chromatin presents a significant barrier to DNA repair. The ability of the DNA repair machinery to gain access to DNA double-strand breaks (DSBs) is dependent on chromatin remodeling complexes. Here, we describe a crucial role for the Tip60 acetyltransferase (KAT5) and the p400 SWI/SNF-related ATPase, sub-units of the NuA4 complex, in the repair of DSBs. NuA4 is rapidly recruited to DSBs, promoting the acetylation of histone H4 by Tip60, which, in combination with p400's ATPase activity, alters the local chromatin structure, creating "relaxed" chromatin domains adjacent to the DSB. These relaxed chromatin, open chromatin structures are required for ubiquitination of the chromatin by RNF8/RNF168 and for the recruitment of other DNA repair proteins, including brca1 and 53BP1, to DSBs.

The recruitment of the NuA4 complex to DSBs loads the acetyltransferase activity of Tip60 and the ATPase of p400 onto the chromatin. The combination of histone acetylation and p400's ATPase activity then decreases nucleosome stability at DSBs and facilitates the recruitment of DNA repair complexes. This critical role for p400's ATPase activity in regulating DNA repair indicates that alterations in chromatin structure are essential elements of the cells response to DNA damage.

Wee1 regulation of the S/M checkpoint

Shelagh D. Campbell [1], Joseph Ayeni[1], Ramya Varadarajan[1] and Ellen Homola

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When cells enter mitosis before chromosomal DNA replication and repair is complete, they undergo lethal mitotic catastrophe. Cell cycle checkpoints normally prevent this from occurring by blocking activation of the major mitotic regulatory kinase Cdk1, during S and G2 phases. The checkpoint mechanism regulates Cdk1 inhibitory phosphorylation, which is catalyzed by Wee1 and Myt1-related kinases. Although Wee1 and Myt1 kinases are Cdk1 inhibitors during S and G2 phases, their relationships change as cells enter mitosis when Cdk1 becomes activated, and inhibits Wee1 and Myt1. Exactly how cells coordinate transitions between these fundamentally different states remains unclear. In human cancers, G1/S checkpointcompromised cells are dependent on Wee1-mediated S/M checkpoint mechanisms to prevent spontaneous DNA damage and mitotic catastrophe. Accordingly, novel chemical inhibitors of Wee1 are being developed to exploit this vulnerability. We are studying the role of Wee1 in a developmental S/M checkpoint mechanism that prevents mitotic catastrophe during early *Drosophila* embryogenesis. Specific aims of our research are 1) to determine the functional significance of Wee1 nuclear localization, 2) to characterize protein-protein interactions important for Wee1 activity and 3) to identify cis-acting motifs that positively regulate Wee1 activity. Our experiments are expected to provide insights into the molecular mechanisms that govern Wee1 activity during embryonic development in *Drosophila*, as well as in humans.

Does telomere length control the lifespan of dog breeds?

Laura J. Fick[1], Mark Li[2]*, Eric Cao[3]*, Bob Bao[3]* and Karl T. Riabowol[1]

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For thousands of years humans have benefited from companionship with the domestic dog (*Canis lupis familiaris)*. Now it appears as though man's best friend may help clarify a pervading question in aging research: Does telomere length reliably dictate longevity within a species? Telomeres are nucleoprotein structures found at the ends of chromosomal DNA that act to protect the ends of the genome from replicative loss and inappropriate DNA repair processing. Dog telomeres are similar in length to human telomeres (~20 kb) indicating that telomere dynamics may be similar between the two species. Further, it is generally accepted that specific genetically isolated dog breeds have characteristic lifespans, making the dog an excellent model to study the impact of telomeres on longevity. Using DNA isolated from the blood of various purebred dog breeds we will use quantitative PCR to measure absolute telomere length. We hypothesize that longer telomeres predict a greater healthy life expectancy. We also hypothesize that dog breeds with shorter telomeres demonstrate a susceptibility to cancer, further providing evidence of the relationship between cancer, lifespan and telomere length. We will also demonstrate evidence of different rates of telomere loss across the breeds and also between the sexes as suggested by preliminary data from studies of human telomere dynamics.

We acknowledge support for this work from AI-HS, CIHR and the Sanofi Aventis BioTalent Challenge.

Pc2 regulates BMI-1 recruitment at sites of DNA damage

Ismail Hassan Ismail[1], Darin McDonald[1], Jean-Philippe Gagné[2], Guy G Poirier[2] and Michael J Hendzel[1]

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Polycomb group proteins (PcG) are major determinants of cell identity, stem cell pleuripotency, and the epigenetic gene silencing involved in cancer development. The Polycomb sumo ligase, Pc2, is phosphorylated by HIPK2 in response to DNA damage, which is thought to control sumoylation of Pc2 itself and to regulate its E3 sumo activity towards HIPK2. Recently numerous PcG, including Pc2, where shown to accumulate at sites of DNA damage. However, it remains unclear whether or not Pc2 is directly involved in the DNA damage response (DDR). Here we define a novel role for Pc2 as an early DDR protein that mediates Sumo conjugation at sites of DNA lesions. DNA damage induces Pc2 to directly sumoylate BMI-1 at lysine 88, which is required for BMI-1 focal accumulation at sites of DNA breaks. Moreover, we establish that Pc2 recruitment to the sites of DNA damage requires PARP activity, but does not require H2AX, RNF8, BMI-1 nor PI-3 related kinases. The importance of Pc2 in the DDR was confirmed by its ability to promote cellular resistance to ionizing radiation. The data elucidates a direct role for Pc2 in the DDR pathway, and suggests potential cross-regulation between polycomb gene silencing and DNA repair functions.

Combined loss of ATM and p53 increases toxicity of PARP inhibitors in Mantle Cell Lymphoma

Chris T. Williamson[1,2], Huong Muzik[2,4], D. Gwyn Bebb[2,3,4] and Susan P. Lees-Miller[1,2,4]

[1]Department of Biochemistry and Molecular Biology, [2]Southern Alberta Cancer Research Institute and [3]Department of Oncology, University of Calgary, Calgary, Alberta, Canada.
[4]Translational Research Laboratory, Tom Baker Cancer Centre, Calgary, Alberta, Canada.

Small molecule inhibitors of PARP (Poly-ADP Ribose Polymerase) have shown promise in the treatment of malignancies deficient in the homologous recombination repair proteins BRCA (breast and ovarian cancer susceptibility) 1 and 2. Furthermore, preclinical studies have demonstrated the potential utility of PARP inhibitors in targeting additional tumour types deficient in other DNA damage repair proteins, such as ATM (Ataxia-Telangiectasia Mutated). Here we show that Mantle Cell Lymphoma (MCL) cells deficient in both ATM and p53 are more sensitive to PARP inhibitors than cells lacking ATM function alone. Moreover, adding single strand break inducing agents further increases the sensitivity of ATM/p53 deficient MCL cells to PARP inhibitors. We demonstrate that PARP inhibition induces DNA dependent protein kinase (DNA-PK)-dependent phosphorylation and stabilization of p53 in ATM-deficient MCL cells, resulting in the expression of p53-responsive cell cycle checkpoint regulators. Lastly, we demonstrate that direct inhibition of DNA-PK results in reduced toxicity of PARP inhibitors in ATM-deficient cells. This study reveals that DNA-PK and p53 play important roles in the response of ATM-deficient MCL cells to PARP inhibitors and suggests that the combination of ATM and PARP inhibitors may be useful in the treatment of p53-deficient malignancies.

Analysis of protein dynamics at active, stalled and collapsed replication forks

Bianca M. Sirbu, Frank B. Couch and David Cortez

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Successful DNA replication and packaging newly synthesized DNA into chromatin is essential to maintain genome integrity. Defects in the DNA template challenge genetic and epigenetic inheritance. Unfortunately, tracking DNA damage responses (DDR), chromatin assembly and maturation at replication forks is difficult in mammalian cells. Here we describe a technology called iPOND (isolation of proteins on nascent DNA) to analyze proteins at active and damaged replication forks at high spatial and temporal resolution. Using this technology, we define the timing of replisome protein recycling and chromatin assembly. Chromatin maturation can continue even when decoupled from replisome movement. Fork stalling causes changes in phosphorylation and ubiquitylation with a switch in the DDR at persistently stalled forks. These data reveal a dynamic recruitment of proteins and post-translational modifications at damaged forks and surrounding chromatin. Furthermore, our studies establish iPOND as a powerful methodology to study DNA replication and chromatin maturation.

Decomplexifying the Smc5-6 complex: From DNA-binding activity to anti-recombination functions

Damien D'Amours

Institute for Research in Immunology and Cancer (IRIC), University of Montreal, Québec, Canada.

The Smc5-6 complex is an essential regulator of genomic stability. Despite this important function, the actual nature of the DNA substrates recognized by the Smc5-6 complex during DNA repair is currently unknown. We have taken a biochemical approach to decipher the key contributions of individual components of the Smc5-6 complex to the overall biochemical activities of the holocomplex. We show here that both Smc5 and Smc6 bind strongly and specifically to single-stranded DNA (ssDNA). Remarkably, the DNA-binding activity of individual Smc proteins does not require the other components of the complex, thereby suggesting that both Smc5 and Smc6 have the ability to target the holocomplex to its DNA substrates *in vivo*. We also demonstrate that Smc5 is able to bind efficiently to oligonucleotides consistent in size with ssDNA intermediates produced during DNA repair. Interestingly, we show that the ATPase activity of the Smc5-6 complex is essential for its functions *in vivo* and that ATP regulates the association of Smc5 with its substrates *in vitro*. Collectively, our data on the DNA-binding activities of Smc5 provide a compelling molecular basis for the role of the Smc5-6 complex in the DNA damage response. Notably, our results suggest a potential mechanism for the putative anti-recombination functions of the complex.

The Smc5/6 complex component Nse5 regulates sumoylation and replication fork stability

Denise E. Bustard[1], Dana Branzei[2], and Jennifer A. Cobb[1]

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The Smc5/6 complex is in the Structural Maintenance of Chromosome (SMC) family of complexes that also includes cohesin and condensin. Within the Smc5/6 complex there are six Non-Smc-Elements (Nse1-6). Here we characterize the role of Nse5 within the complex and during DNA replication. A temperature sensitive mutant called *nse5*-ts shows a marked reduction in Smc5 and Yku70 sumoylation and additive sensitivity to HU and MMS-induced replication stress when combined with *mms21*-11, a catalytically dead version of the E3 small ubiquitin modifier (SUMO) ligase, Mms21 (Nse2). Nse5 physically interacts with SUMO and the E2 conjugating enzyme Ubc9 through two putative SUMO Interacting Motifs (SIMs), and these interactions are important for facilitating the sumoylation of Smc5. Quite surprisingly, mutations in the SIMs did not lead to DNA damage sensitivity or show defects at stalled replication forks despite a loss of Smc5 sumoylation. These data uncouple the important function of the Smc5/6 complex during replication stress from the sumoylation of Smc5. Much like Mms21, we suggest the essential function of Nse5 is not its function in the SUMO pathway, but rather its interaction within the Smc5/6 complex.

Srs2: Guardian of lagging strand DNA replication

Linda J. Reha-Krantz, Md. Shahjahan P. Siddique and Ashlesha Sonpar

Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada.

There is a division of labor at eukaryotic DNA replication forks with DNA polymerase ε performing leading strand replication and DNA polymerase δ serving as the lagging strand DNA polymerase (1). We have engineered a mutant DNA polymerase δ in *Saccharomyces cerevisiae* (the L612M-DNA pol δ) that is sensitive to the antiviral drug phosphonoacetic acid (PAA) (2); thus, PAA can be used to selectively compromise lagging strand replication in cells expressing the mutant DNA polymerase. PAA triggers the S-phase DNA damage checkpoint in *pol3-L612M* cells as demonstrated by production of phosphorylated Rad53 (Chk2) that is dependent on Rad9, but not Mrc1; phosphorylated γH2AX is also detected (3). Generally, post replication repair (PRR) is needed for cells to recover or adapt to DNA damage, but PAA-treated *pol3-L612M* cells do not need PRR components that depend on mono or poly ubiquitylated PCNA. In fact the opposite is true; deletion of *MMS2*, which is required for production of poly ubiquitylated PCNA, reduces PAA-sensitivity. However, deletion of the *SRS2* helicase gene confers severe PAA-sensitivity. We propose that Srs2 function is needed to prevent production of aberrant recombination intermediates that arise if recombination is used to repair persisting gaps in the lagging strand of PAA-treated *pol3-L612M* cells.

This work was funded by CIHR and AHFMR.

(1) Nick McElhinny *et al.* (2008) *Mol. Cell* 30: 137
(2) Li *et al.* (2005) *Genetics* 170: 569
(3) Reha-Krantz *et al.* (submitted)

Mechanisms for maintaining genome stability at the replication fork

Karlene A. Cimprich

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Maintenance of genome stability is of critical importance to the cell, and the integrity of the genome can be threatened when DNA is damaged by either endogenous or environmental agents. DNA is particularly susceptible to damage during DNA replication, when replication forks can stall at DNA lesions. As a result, the cell has finely tuned processes to allow the replication of damaged DNA, to repair the DNA damage and to stabilize/restart stalled replication forks. A failure to carry out these processes can lead to the loss of genomic integrity by interfering with proper repair of DNA, and by leading to the collapse of stalled forks into double-strand breaks. We carried out a genome-wide screen to identify proteins involved in preventing DNA damage in the absence and presence of replication stress. Our analysis revealed unexpected connections between known cellular pathways and the prevention of DNA damage. Our recent progress on the proteins identified in this screen and their roles in maintaining genome stability will be described here. Furthermore, we will describe recent studies regarding the role of other proteins known to promote the replication of damaged DNA either by direct bypass or via restart of DNA replication.

Linking nuclear DNA damage and epigenetic changes to stem-cell decline during aging

Margarida Santos, Kai Ge, Andre Nussenzweig

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Aging is accompanied by a myriad of organismal changes including a declining innate and adaptive immune system, increased bone marrow failure, myleloid proliferative diseases and cancer. These pathologies arise, at least in part from age dependent decline in hematopoietic stem cell (HSC) function. Compared to younger stem cells, HSCs from old mice show increased accumulation of reactive oxygen species, DNA damage, and loss of epigenetic regulation leading to altered gene expression. The mechanisms underlying increased DNA damage, diminished lymphopoiesis and enhanced myelopoiesis, which might contribute to increased incidence of leukemia in the elderly, remains unclear. Known epigenetic gene regulators of HSC function include Ezh2 and Bmi1, which are members of the Polycomb family of transcription factors, and the mixed lineage-leukemia protein MLL1, which is part of the SET domain containing group of histone H3K4 methyltransferase complexes. The only MLL-associated factors that are reported to be altered in aged stem cells are the MLL3 complex members-MLL3, PTIP and PA1- all of which were down regulated. Recently we showed that PTIP plays a dual role in B lymphocyte specific class-switch recombination, by regulating H3K4me3 dependent transcription as well as promoting DNA repair at immunoglobulin genes. We are currently exploring the role of additional MLL3/MLL4 complex members (MLL3, MLL4, PTIP, PAI, NCOA6 and UTX) in HSC function. Our data suggests that PTIP conditional knockout mice show defects in stem cell function, evidenced by a severe decrease of the lineage-negative Sca-1+, c-Kit+ (LSK) compartment, defective repopulating activity, and a myeloproliferative syndrome with splenomegaly. In the absence of MLL4, HSCs are present there is a skewing towards myeloid differentiation and signs of myeloproliferative disease. Moreover, absence of PTIP or MLL4 causes elevated levels of reactive oxygen species in the HSCs, persistent DNA damage, cell-cycle dysregulation and dramatic defects in lymphoid precursor cells. In summary, our work suggests that mis-regulation of PTIP dependent H3K4me3 gene expression or DNA repair may be one of the mechanisms underlying age-dependent stem cell decline and the unbalanced expansion of myeloid progenitor cells. This provides further evidence for interplay between DNA damage, epigenetic changes and metabolic stress.

Regulation of the mitotic checkpoint – RZZ complex

Gordon Chan

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The RZZ complex, an essential component of the mitotic checkpoint, consists of Rod, Zw10 and Zwilch. The hRZZ complex localizes to kinetochores in prometaphase and diminishes during metaphase at which time it is found on the spindle and spindle poles. We found that hZw10 residency at metaphase kinetochores is brief. However, during prometaphase or at unattached kinetochores, hZw10 becomes a stable component of the kinetochore. This is a unique feature for a kinetochore protein as all other components are either stable or dynamic at the kinetochore. Moreover, we find that stable hZw10 kinetochore residency at prometaphase kinetochores is dependent on its interaction with hZwint-1, and is essential for the mitotic checkpoint.

The mitotic checkpoint ensures accurate chromosome segregation by monitoring microtubule-kinetochore interaction and kinetochore tension. Recently, we found that the RZZ complex is accumulated at tensionless kinetochores in an Aurora B kinase dependent fashion. Therefore, we examined whether these proteins are phosphorylated and if it has an effect on their kinetochore localization and function. We found that Aurora B kinase mediated phosphorylation of hZwint-1 promotes interaction with hZw10. The phosphorylation of hZwint-1 by Aurora B kinase may be involved in the stabilization of hZw10 at prometaphase and tensionless kinetochores.

Ubiquitin recognition and remodeling at DNA double strand breaks

Roger A. Greenberg

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Germline mutations to the Breast Cancer Early Onset 1 gene (BRCA1) confer a strong predisposition to breast and ovarian epithelial cancers. BRCA1 maintains genomic integrity by activating cell cycle checkpoints and error-free mechanisms of DNA double strand break (DSB) repair. Cancer causing mutations disrupt these activities primarily by impairing BRCA1 recognition and retention at DSBs. We have discovered that BRCA1 is targeted to DSBs via an interaction with the ubiquitin interacting protein, RAP80 (Sobhian et al. *Science* 2007). RAP80 provides a DNA damage recognition element by specifically binding lysine^{63}-linked ubiquitinated (K63-Ub) structures on chromatin adjacent to DSBs for a complex consisting of BRCA1 and several other proteins (Shao et al. *Genes & Development* 2009; Shao et al. *PNAS* 2009). These results imply that ubiquitin recognition is required for genome integrity and suppression of malignancy. Related to this hypothesis, new insights into the roles of this complex in DNA repair and tumor suppression will be presented. Recent findings from my laboratory have revealed that DSB associated ubiquitin also enables communication between DNA damage responses and transcriptional processes that occur on chromatin in cis to the site of damage (Shanbhag et al. *Cell* 2010). Using a novel assay that we have developed for single cell analysis, we have observed an ATM dependent process that silences transcription for multiple kilobases in cis to DSBs. New mechanistic insights into this ATM dependent DSB silencing pathway will be presented along with its implications to DSB repair, epigenetic inheritence, carcinogenesis, and viral latency.

The spindle assembly factor TPX2 regulates DNA damage response via MDC1 / ATM-dependent amplification of p-H2AX

Gernot Neumayer[1,2], Su Yeon Shim[1,2], Hoa Le Thi[1,2], Cecilia Lundin[4], Camille Belzil[1], Mathieu Chansard[1], Yaping Yu[2], Yulan Jiang[1,2], Oliver Gruss[3], Susan P. Lees-Miller[2], Thomas Helleday[4] and Minh Dang Nguyen[1,2]

[1]Departments of Clinical Neurosciences and Cell Biology & Anatomy, Hotchkiss Brain Institute and [2]Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Canada. [3]DKFZ-ZMBH Alliance, ZMBH, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany. [4]Gray Institute for Radiation Oncology and Biology, Radiobiology Research Institute, Churchill Hospital, Oxford OX3 7LJ University of Oxford, UK.

The microtubule-associated protein TPX2 is required for spindle formation and mitosis. In interphase, TPX2 is imported to the nucleus to prevent its premature microtubule organization activity. No function has been assigned to nuclear TPX2. We now report that TPX2 is involved in the response to DNA double strand breaks (DSB) induced by ionizing radiation (IR). TPX2 interacts with MDC1 to regulate the MDC1/ATM-dependent amplification of p-H2AX. Loss of TPX2 leads to inordinately strong p-H2AX accumulation in G0 and G1 and the increased formation of high intensity p-H2AX foci. Pharmacologic inhibition or depletion of ATM, but not of DNA-PK, rescues this phenotype. Moreover, cells lacking TPX2 have defects in recruitment and disengagement of 53BP1 and RAD51, respectively at the sites of lesions, accumulate more DSBs and ultimately, undergo increased apoptosis. Conversely, cells overexpressing TPX2 have reduced levels of p-H2AX after IR. Critically, the regulation of p-H2AX signals by TPX2 is independent from TPX2's mitotic roles as confirmed by gain and loss of function experiments in post-mitotic neurons that have permanently exited the cell cycle. In conclusion, our study unravels a novel and the first nuclear function for TPX2 in the cellular responses to DNA damage.

KAP-1 phosphorylation regulates heterochromatic CHD3 nucleosome remodeling during the DNA double strand break response

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KAP-1 poses a significant barrier to DNA double strand break (DSB) repair within heterochromatin (HC) which is alleviated by ATM dependent KAP-1 phosphorylation (pKAP-1). Here, we address the mechanistic consequences of pKAP-1 that promote HC DSB repair and chromatin relaxation. KAP-1 function involves autoSUMOylation and subsequent recruitment of nucleosome deacetylation, methylation and remodeling activities. While significant changes in HC acetylation or methylation were not detected, radiation induced pKAP-1 dispersed the nucleosome remodeling enzyme CHD3 from DSBs and triggered concomitant chromatin relaxation; pKAP-1 loss rapidly reversed these effects. Importantly, CHD3 depletion, catalytic inactivation or ablation of its interaction with KAP-1^{SUMO1} bypassed pKAP-1's role in repair. Although KAP-1 SUMOylation was unaffected after irradiation, CHD3 dissociated from KAP-1^{SUMO1} in a pKAP-1 dependent manner. We provide evidence that KAP-1^{S824} phosphorylation generates a motif that directly perturbs interactions between CHD3's SUMO interacting motif and SUMO1, dispersing CHD3 from HC DSBs and thereby enabling repair.

Insights into the mechanism of action of nonclassical platinum–acridine anticancer agents from chemogenomic screening

Kahlin Cheong-Ong[1], Kevin Song[1], Zhidong Ma[2], Daniel Shabtai[1], Ulrich Bierbach[2], Guri Giaever[1] and Corey Nislow[1]

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Platinum-based drugs are among the most successful drugs currently in use in cancer chemotherapy. The most widely used platinum chemotherapeutic, cisplatin (*cis*-diamminedichloroplatinum(II)), acts by forming cross-links (mainly intrastrand) in the major groove of DNA, which leads to inhibition of DNA synthesis and RNA transcription, cell cycle arrest and, ultimately, activation of the apoptosis machinery. Although cisplatin is effective against diverse cancers, limitations to its clinical use include dose-limiting toxicites and the frequent emergence of drug resistance. We have developed a class of non-classical platinum-based compound comprised of platinum-acridine conjugates. These agents were developed as molecules that combine the DNA-damaging features of a platinum complex with the DNA intercalating properties of acridine-based chromophores. They manifest a novel mechanism of DNA damage independent of DNA crosslinking. To better characterize the mechanism of action of these agents, we used a validated, full genome chemical genomic assay to examine a small library of these hybrid agents along with their platinum-free carrier ligands. We find that four of the platinum-acridine compounds show unambiguous DNA-damaging effects and that one compound in particular, PT-AMIDINE, produces a unique DNA-damage response profile.

Characterization of Dbf4 interactions with DNA replication and checkpoint factors

Matthew D. Ramer[1], Darryl R. Jones[1], Lindsay A. Matthews[2], Ajai A. Prasad[1], Alba Guarné[2] and Bernard P. Duncker[1]

[1]Department of Biology, University of Waterloo, Waterloo, ON, Canada. [2]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada.

Dbf4/Cdc7 kinase (DDK) phosphorylates the Mcm2-7 helicase complex, triggering the initiation of DNA replication. Dbf4 contains three conserved regions, motifs N, M and C. Motif C includes a CCHH-type zinc finger, and we previously demonstrated that replacement of conserved amino acids within this region decreases Dbf4-Mcm complex association, impairs S-phase progression and increases sensitivity to genotoxic agents. To investigate the DDK-Mcm interaction in greater detail, we performed two-hybrid and co-immunoprecipitation analyses which revealed that Dbf4 and Cdc7 bind to mutually exclusive subsets of the Mcm2-7 subunits. Disruption of either the Dbf4-Mcm2 or Cdc7-Mcm4 interaction compromised growth, suggesting that both are required for efficient targeting of DDK to the Mcm complex. Previously, we showed that motif N mediates an interaction with Rad53, and that Dbf4ΔN cells are hypersensitive to DNA damaging agents. We have determined the crystal structure of a Dbf4 fragment encompassing motif N and found that it adopts a modified BRCT fold with an unusually long helix α1, and an additional N terminal α helix, which we have termed the helix-BRCT (HBRCT) domain. The integrity of the HBRCT domain is essential to preserve the interaction with Rad53, and its disruption explains the checkpoint phenotypes of numerous Dbf4 mutants.

Mechanistic insights on DNA recognition of PNKP phosphatase domain

Nicolas Coquelle, Zahra Havali, Nina K. Bernstein and J.N. Mark Glover

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Many DNA repair strategies have evolved to maintain our genome integrity. In the final steps of many repair pathways, damaged DNA ends are extended by DNA polymerases and sealed by DNA ligases. However, to fulfill these functions, the DNA ends must bear the requisite 5'-P and 3'-OH termini. Through its two catalytic activities (5'-kinase and 3'-phosphatase), polynucleotide kinase phosphate processes single or double stranded DNA with abnormal ends to restore ligatable termini.

The phosphatase activity of PNKP is thought to be more critical for PNKP-dependent DNA repair pathways but until now, little functional and structural data have been available for DNA recognition of PNKP phosphatase domain. Using a catalytically inactive mutant of PNKP, we have recently crystallized and determined the structure of PNKP bound to different single-stranded DNA phosphatase substrates. These structures reveal that the 3'-phosphate binds in a deep narrow cleft. To probe the importance of key residues on DNA substrates recognition, a series of mutant were constructed and their ability to bind different substrates was tested. Intriguingly, phosphatase domain of PNKP also acts on double-stranded DNA (dsDNA), however the DNA binding cleft is too narrow to accommodate these DNAs. We suggest that the PNKP phosphatase domain destabilizes base pairing in dsDNA substrates to facilitate 3' dephosphorylation.

A genome-wide view of protein dynamics during replication stress in *S. cerevisiae*

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In *S. cerevisiae* a number of proteins involved in the DNA damage response relocalize within the cell following exposure to DNA damage or DNA replication stress. We used the collection of *S. cerevisiae* strains in which ~4500 genes, representing 75% of the genome, have been fused to GFP to systematically screen for proteins whose abundance or localization changes in the presence of DNA replication stress caused by MMS or HU. We note localization changes for 253 proteins and abundance changes for greater than 700 proteins. Most abundance changes in response to MMS do not correlate with transcriptional up-regulation, suggesting that the bulk of the MMS response is post-transcriptional even for proteins whose abundance changes. Categorizing proteins by the type of localization change reveals enrichments of GO functions, and enrichment for protein-protein interactions. For example, the group of proteins that localize to cytoplasmic foci upon replication stress was enriched for RNA processing, and in particular indicated an unrecognized role for the LSM complex, a regulator of translation and transcript stability, in the replication stress response. Our data indicate that analysis of protein dynamics following chemical perturbation will be a powerful tool in elucidating response pathways.

DNA replication stress results in expansion of dNTP pools and a mutator phenotype

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The integrity of the genome depends on diverse pathways that regulate DNA metabolism. Defects in these pathways result in genome instability, a hallmark of cancer. Deletion of ELG1 in budding yeast, when combined with hypomorphic alleles of PCNA results in significant amounts of endogenous DNA damage during S phase that elicits upregulation of RNR activity. This leads to a dramatic expansion of dNTP pools in G1 that allows cells to synthesize significant fractions of the genome in the presence of HU in the subsequent S-phase. Consistent with the recognized correlation between dNTP levels and spontaneous mutation, compromising ELG1 and PCNA results in a significant increase in mutation rates. Together, our data point to a vicious circle in which mutations in gatekeeper genes give rise to genomic instability during S phase, which in turn results in high levels of spontaneous mutagenesis.

The MRX complex regulates cohesin during replication: In times of stress keep your sister close

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The Mre11-Rad50-Xrs2 (MRX) complex is important for the maintenance of genomic integrity and is recruited to forks during pauses in replication. One essential function of the MRX complex is to provide architectural support to DNA during repair, meiotic recombination and telomere maintenance. Structural analysis of the MRX complex suggests that it can bridge two strands of duplexed DNA and serve as a long-range tether between sister chromatids. Moreover, a dramatic loss of cell viability in the presence of the DNA replication inhibitor HU is observed when disruptions in MRX are combined with mutations in factors involved in sister chromatid cohesion (SCC). Establishment of SCC is coupled to replication fork progression and is facilitated by the cohesin complex which holds replicated chromosomes together from their synthesis until the onset of anaphase. Using ChIP-Chip analysis we show increased cohesin association with forks when they stall, above levels detected in an unperturbed S phase, and this is dependent on the MRX complex. In addition, we find the MRX complex is necessary for proper sister chromatid cohesion (SCC), and this function depends on the structural integrity and bridging capacity of Rad50. Taken together, our data suggests one critical role for the MRX complex in preserving chromosome integrity involves supporting the architecture of newly synthesized daughter strands during pauses in replication, preventing fork-associated damage.

Functional interdependence of lysine acetylase Rtt109 and histone chaperone Asf1 in transcriptional regulation

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Lysine acetylase Rtt109 and histone chaperone Asf1 directly or indirectly regulate a plethora of DNA-dependent reactions in the nucleus, some of which contribute to the control of genome instability and ageing. Here we examine Asf1 regulation of chromatin by mechanisms that either favor or disfavor transcription. In particular, we examine how Asf1 switches its control of chromatin between activation and repression of *ARG1*, a metabolic gene in yeast. Under conditions that repress *ARG1*, an activating function of Asf1 is overridden by Asf1 inhibition that requires its binding to H3/H4. The H3 K56 acetylase Rtt109 is enriched at the *ARG1* promoter under repressing conditions and suppresses the activating function of Asf1 by a mechanism that does not involve H3 K56 acetylation. On the other hand, H3 K56 acetylation by Rtt109 acting in concert with Asf1 accounts for their stimulation of *ARG1* under inducing conditions. Therefore Asf1 is the cofactor for histone acetylation by Rtt109 and the target of an Rtt109-dependent switch mechanism that controls its effector function in transcriptional regulation.

Ubiquitin signalling orchestrates the response to DNA double-strand breaks

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Our knowledge of the response to DNA double-strand breaks (DSBs) originates primarily from genetic studies in genetically-tractable organisms or via the positional cloning of genes responsible for chromosomal instability syndromes. We have recently implemented a sensitive cell-based high-throughput assay that detects the formation of 53BP1 ionizing radiation-induced foci (IRIF). With this assay, we have completed a first pass genome-wide siRNA screen. I will report the results of this and I will focus on our identification of a regulatory ubiquitylation cascade that is composed of RNF8 and RNF168, two E3 ubiquitin ligases that promote the focal accumulation of 53BP1 and BRCA1 to sites of DNA damage. In my talk, I will focus on our latest work directed towards understanding the regulation of the RNF8/RNF168 pathway and in particular the contribution of negative feedback loops. One example that we have recently uncovered is the non-canonical action of the deubiquitylating enzyme OTUB1 on this pathway. Finally, it is important to note that biallelic mutations in the RNF168 gene are responsible for the recently described RIDDLE immunodeficiency and radiosensitivity syndrome. RNF8 and RNF168 therefore define a novel branch of the DNA damage response that is important for human physiology.

Novel chromatin remodeling factors that promote signaling and repair of DNA damage

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Ionizing radiation (IR)-induced DNA double-strand breaks (DSBs) provoke a multitude of cellular responses, including cell cycle checkpoints and DNA repair. How cells signal and repair DSBs within chromatin is poorly understood. We use RNAi-based screens in worms and human cells to identify novel factors that orchestrate the DSB response in the context of chromatin. Through these screens we have identified several factors, including human CHD4 and SMARCA5/SNF2h, which are the catalytic subunits (ATPases) of functionally distinct chromatin remodeling complexes. We show that depletion of these ATPases leads to increased IR sensitivity. Both CHD4 and SMARCA5 accumulate in DSB-containing chromatin tracks generated by laser micro-irradiation. Directly at DSBs, they promote RNF8/RNF168-dependent formation of ubiquitin conjugates to mediate the accumulation of RNF168 and BRCA1. In addition, loss of these ATPases impairs G2/M checkpoint responses and DSB repair. Interestingly, knockdown of SMARCA5 dramatically increases the sensitivity to PARP inhibition, suggesting that SMARCA5 could serve as an important novel target in cancer therapy. Taken together, we show that the CHD4 and SMARCA5 chromatin remodelers are novel factors that orchestrate signaling and repair of DSBs, preserve genome integrity and may suppress cancer development.

Polycomb group proteins in the DNA damage response

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We and others have recently reported that the polycomb repressor complex 1 (PRC1 complex), which is a histone H2A E3 ubiquitin ligase, is recruited to sites of DNA double-strand breaks and laser micro-irradiation, where it participates in the ubiquitylation of histone H2A and H2AX. This polycomb group repressor complex has a well-established role in transcriptional silencing during development and may have a similar function in the repair of DNA double-strand breaks, which involves transcriptional silencing of the chromatin surrounding the DNA damage site. I will present new information on additional biochemical activities that are associated with the polycomb group family of proteins (histone methylation, sumoylation) and their importance in DNA repair. I will also describe further experiments detailing the mechanisms of recruiting polycomb group proteins to sites of DNA damage. Consistent with this being a separate and parallel pathway to the previously described RNF8 pathway, we find a requirement for sumoylation in the recruitment of the PRC1 proteins to sites of DNA damage but this requirement involves different sumo ligases than the PIAS1 and PIAS4, which have been reported to be important for RNF8 function. The implication of these results in the radiation resistance of cancer stem cells will also be discussed.

Prolactin induces breast cancer cell resistance to DNA damaging agents via a heat shock protein-90-mediated mechanism

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Prolactin can function in an endocrine and autocrine/paracrine manner to initiate mammary tumors (rodents), increase the risk of human breast cancer, increase cancer progression, and increase the survival of breast cancer cells. Evidence also points to a role for prolactin in the chemotoxic resistance of breast cancer cells, and we have evidence to suggest that prolactin can induce cellular resistance to DNA damaging agents through a molecular mechanism that involves heat shock protein-90 (HSP90). We identified that the inducible isoform, HSP90alpha, is a prolactin-JAK2-STAT5B target gene using gene expression profiling and reporter assays. Cell viability assays demonstrated that prolactin increases cell viability of DNA damaged cells. The increase in cellular resistance despite DNA damage is specific to the PRL receptor, as the PRL receptor antagonist, Δ1-9-G129R-hPRLR, completely reduced the increase in viability. A HSP90 inhibitor completely abolished the PRL-mediated increase in cell viability of doxorubicin treated cells. We are exploring the role of a HSP90 client protein that is involved in the DNA damage response, in this mechanism. In conclusion, PRL confers resistance of breast cancer cells to DNA damaging chemotherapeutics by a HSP90-dependent mechanism.

Structural insights into the mechanism of nonhomologous end-joining

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Non-homologous end joining (NHEJ) is the major pathway for the repair of ionizing radiation (IR) induced DNA double strand breaks (DSBs) in mammalian cells. We have used small angle X-ray scattering and crystallography to determine the structures of key NHEJ components: Ku70/80 heterodimer, DNA-PKcs, XRCC4, XLF and the BRCT domain of DNA ligase IV. We show that autophosphorylation of DNA-PKcs induces a large conformational change that we propose opens the gap at the base of the molecule, facilitating release from DNA-bound Ku. The crystal structure of the XRCC4-XLF complex reveals that L115 in the head domain of XLF fits into a preformed pocket in the head domain of XRCC4, confirming the solution structure of the complex by SAXS. Significantly, XRCC4-XLF form long super-helical filaments that interact in parallel to form a U-shaped alignment channel that accommodates dsDNA to facilitate ligation by DNA ligase IV. The implications of these structures for NHEJ will be discussed.

ABSTRACTS

POSTER PRESENTATIONS

POSTER #1

Cellular orchestration of the DNA single-strand break repair pathway

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Cellular DNA is continually threatened by intracellular and extracellular agents. Single-strand breaks (SSBs) comprise one of the most frequent forms of DNA damage. Consequently a well established SSB repair (SSBR) pathway exists, in which PARP-1 catalyzes the formation of poly(ADP-ribose) (PAR) chains, which serve (1) to flag the break for downstream proteins and (2) improve access of SSBR proteins to the site of damage. Once the PAR residues are established, the SSBR core machinery is recruited and the repair process ensues. The first step is to clean up the DNA ends because DNA damaging agents often produce strand break termini that are incompatible with gap filling and ligation steps. One of the most versatile DNA end processing enzymes is PNKP. Once proper DNA ends are restored gap filling is carried out by DNA polβ and finally DNA ligase IIIα seals the nick. A major player that helps coordinating the steps from end processing to nick sealing is XRCC1. Most of our current understanding of SSBR has come from *in vitro* biochemical studies, but less is known about the process occurring inside the cell. Accordingly we employed live cell imaging approach to study the SSBR pathway in live cells.

POSTER #2

XRCC4/DNA ligase IV enhances polynucleotide kinase/phosphatase kinase activity via a novel phosphorylation-independent interaction

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If unrepaired, or misrepaired, DNA single- and double-strand breaks (SSBs and DSBs) drive chromosomal instability that can lead to either cell death or neoplastic transformation. The enzyme polynucleotide kinase/phosphatase (PNKP) participates in both base excision repair and non-homologous end-joining (NHEJ) to repair SSBs and DSBs, respectively. In these mechanisms, PNKP is the primary enzyme for processing abnormal 5'-hydroxyl and 3'-phosphate ends that preclude the final enzymatic repair steps by DNA polymerases and ligases. At the lesion, the PNKP forkhead-associated (FHA) domain interacts with a phosphorylated motif on its partner protein and uses dual 5'-DNA kinase/3'-DNA phosphatase activities to restore requisite 5'-phosphate and 3'-hydroxyl ends. In NHEJ, PNKP forms a complex with 2:1 XRCC4/DNA Ligase IV (LigIV) heterodimer to achieve this biological role. I have generated high quality models of the PNKP/XRCC4/LigIV complex and its components. The SAXS results suggest a secondary interaction between PNKP and XRCC/LigIV beyond the FHA:cognate phosphopeptide. This is supported by the non-equimolar stoichiometry of 1 PNKP:2 XRCC4:1 LigIV in the complex, as shown by multi-angle laser light scattering (MALLS) results. I have shown the functional implications for this interaction, which show that the PNKP kinase activity on DNA substrates increases upon binding to XRCC4/LigIV.

POSTER #3

Low dose radiation effects: A preliminary investigation of dose- and irradiation regime-dependent effects on gene expression

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Low dose radiation (LDR) effects upon the genome are becoming increasingly important to elucidate, especially in higher levels of biological organization. Irradiation is known to cause genome instability, and both genetic and epigenetic (DNA methylation, RNA-mediated silencing and histone modification) mechanisms work to regulate genome stability and gene expression. Specific LDR-induced alterations in gene expression are relatively unknown. Also, effects due to exposure regime differences and the persistence of these changes are unclear. In this study, mature C57Bl/6 male mice were irradiated and separated into six cohorts: 0 Gy, 1 Gy, 0.1 Gy, 0.01 Gy, 0.01 Gyx10 (fractionated dose) and 0.01 Gy+1 Gy ("priming" and subsequent "challenge" dose). Each cohort contained eighteen mice, divided equally between three sacrificial time points (6 hours, 96 hours and 4 weeks after completion of radiation regime) to determine alteration persistence. Liver tissue was collected and DNA, RNA and protein samples were isolated. The Illumina platform for whole-genome expression revealed that LDR exposure caused alterations in expression of multiple genes between cohorts and time points. Combined with epigenetic data, it is anticipated that low dose irradiation will alter genome profiles and impact radiation target organs of the exposed animals in time- and dose-dependent manners.

POSTER #4

Nucleases are required for DNA post-replication repair

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In *Saccharomyces cerevisiae* DNA post-replication repair (PRR) functions to bypass replication-blocking lesions in order to maintain genomic integrity and prevent damage-induced cell death. PRR employs two different mechanisms to bypass damaged DNA; translesion synthesis (TLS) and error-free PRR. We recently demonstrated that error-free PRR utilizes homologous recombination (HR) to facilitate template switching and bypass DNA lesions. Null mutations of HR genes including *rad51, 52, 54, 55* and *57* are known to confer characteristic synergistic interactions with TLS mutations. To our surprise, null mutations of genes encoding the Mre11-Rad50-Xrs2 (MRX) complex, which is also required for HR, are epistatic to TLS mutations. The MRX complex confers an endo/exonuclease activity required for the detection and processing of DNA double-strand breaks (DSBs). Our results suggest that the MRX complex functions in both TLS and error-free PRR and that this function requires the nuclease activity of Mre11. This is in sharp contrast to other known HR genes that only function downstream of error-free PRR. Furthermore, we found that inactivation of *SGS1* significantly inhibits PCNA mono-ubiquitination and is epistatic to mutations in TLS, suggesting that Sgs1 also functions at earlier steps in DNA lesion bypass. We also examined roles of Sae2 and Exo1, two accessory nucleases involved in DSB resection in PRR. We found that while Sae2 is primarily required for TLS, Exo1 is exclusively involved in error-free PRR. In light of the distinct and overlapping activities of the above nucleases in the resection of DSBs, we propose that the distinct single-strand nuclease activities of MRX, Sae2 and Exo1 dictate the preference between TLS and error-free PRR for lesion bypass.

POSTER #5

Modulation of drug-induced DNA damage signaling by sodium salicylate and non-steroidal anti-inflammatory drugs (NSAIDs)

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In response to doxorubicin, cells robustly activate DNA damage signaling pathways, as monitored by phosphorylation of ATM and its downstream effectors. We had previously observed that this response could be attenuated by pretreatment with N-acetyl cysteine and attributed this to the scavenging of hydroxyl radicals. In a subsequent study, we identified that several putative antioxidants, including sodium benzoate and sodium salicylate, the primary metabolite of aspirin, mimicked this effect. However, this effect was independent of hydroxyl radicals and we have identified sodium salicylate as a novel catalytic inhibitor of human DNA topoisomerase II□. Importantly, we observed that salicylate prevents doxorubicin-induced DNA double-strand break formation by preventing stabilization of the topo II-DNA cleavable complex. As a consequence, salicylate attenuates doxorubicin and etoposide cytotoxicity.

Given the widespread use of aspirin and related non-steroidal anti-inflammatory drugs (NSAIDs), including in the treatment of mild-to-moderate cancer pain, these observations could have clinical implications on the co-administration of NSAIDs during chemotherapy. To expand our understanding of this effect, we are undertaking a systematic evaluation of 1) structural derivatives of salicylates with an aim to identify critical substitutions modulating the observed effects, and 2) evaluating whether similar effects are observed with both salicylate- and non-salicylate based NSAIDs.

POSTER #6

Nse5 regulates sumoylation and replication fork stability

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The Smc5-Smc6 complex is in the Structural Maintenance of Chromosome (SMC) family that also includes the cohesin and condensin complexes. While cohesin and condensin play well-characterized roles in the structure and organization of chromosomes, Smc5-Smc6 plays a poorly understood role in the DNA damage response. Within the Smc5-Smc6 complex there are six Non-Smc-Elements (Nse1-6), of which Mms21 (Nse2) is an E3 small ubiquitin modifier (SUMO) ligase. We have characterized the budding yeast Nse5 component of the complex using a temperature sensitive allele *nse5*-ts. While *nse5*-ts genetically interacts with mutants of the SUMO pathway, the Nse5 protein physically interacts with SUMO and the E2 conjugating enzyme Ubc9. These interactions are important for facilitating sumoylation of Mms21 targets Smc5 and Yku70, and are mediated through two SUMO Interacting Motifs. Much like Mms21, the essential function of Nse5 does not appear to be its SUMO function, but rather in its interaction with the Smc5-Smc6 complex. We have also characterized a role for Nse5 in stabilizing stalled replication forks and resolving Holliday junction-shaped DNA structures that arise when forks collapse. Regulation of these functions is independent of Nse5's function in facilitating sumoylation, and may reflect a need for the structural integrity of the Smc5-Smc6 complex.

POSTER #7

Virtual Screening as a tool to identify inhibitors of the tumour suppressor BRCA1

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Here we explore the possibility of Breast Cancer Associated 1 (BRCA1) as a possible therapeutic target. As a key component of the DNA damage response, germline mutations in BRCA1 have been identified as a major initiator of hereditary breast and ovarian cancers. Conversely, in many sporadic breast cancers, BRCA1 is fully functional and may play a key role in acquired resistance to chemotherapies due to its important role in DNA repair and checkpoint control. The ability to impair these pathways by inhibiting BRCA1 in cancer cells may prove to be a useful means to sensitize tumours to DNA damaging agents. Because BRCA1 deficiencies are only associated with a marked increased risk for cancers in the breast and ovarian tissues, highly specific BRCA1 inhibitors might selectively target these tissues, thereby reducing off-target side effects. Currently, the inhibition of ssDNA repair via PARP1 inhibitors, in addition to the already impaired ability of $BRCA1^-$ tumours to repair dsDNA breaks, have shown to be very effective. In $BRCA1^+$ cancers, a combination of PARP1 and BRCA1 inhibitors may prove to be equally as effective. We use virtual screening coupled with Fluorescence Polarization (FP) and streptavidin pull-down competition assays to locate and verify novel inhibitors of BRCA1.

POSTER #8

A role for PRC2 in the DNA damage response

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Polycomb group proteins (PcG) are chromatin-associated proteins involved in stem cell maintenance and gene repression. Enhancer of Zeste Homolog 2 (Ezh2) is a histone methyltransferase associated with polycomb repressive complex 2 (PRC2) and is best known for the trimethylation of histone 3 lysine 27 (H3K27me3). Overexpression of Ezh2 correlates with aggressive and metastatic tumor profiles, but whether or not this results directly from PRC2-dependent changes in gene regulation remains unknown. In this study, we examine the potential involvement of PRC2 in the DNA damage response (DDR) pathway. GFP labeled Ezh2 is recruited to laser induced DNA damage with similar kinetics as other recognized DDR proteins. Consistent with its function as a DDR protein, knockdown of Ezh2 causes increased sensitivity to gamma-irradiation and reduced ability to repair double-stranded-breaks. Collectively, this data implicates PRC2 in the DDR pathway and PcG in the repair of double-strand-breaks.

POSTER #9

Alterations and effects of microRNA-34a in Hutchinson-Gilford progeria syndrome

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare, segmental premature aging disorder characterized by muscle and skin atrophy and poor growth, osteoporosis, cardiovascular complications and cataracts among other symptoms. Persons afflicted die at a mean age of 13.5 years. At the cellular level, fibroblast cells from HGPS patients also show accelerated aging, diminished proliferative ability and rapidly reach replicative senescence. The cause of HGPS is a sporadic, single base change in one allele of the lamin A gene that alters splicing, resulting in the formation of progerin, a truncated form of lamin A that retains a farnesyl group at its carboxy terminus. The molecular pathways affected by this mutation of lamin A that transduce the HGPS phenotype are currently unknown but involve altered chromatin structure and gene expression including down regulation of the ING1 epigenetic regulator and type II tumour suppressor.

MicroRNAs (miRNAs) are small and non-coding RNA molecules that have the ability to regulate the levels of multiple gene transcripts. They are important gene regulators in cellular pathways such as proliferation, apoptosis and senescence. MicroRNA-34a (miR-34a) was reported to inhibit cell proliferation, induce apoptosis and induce senescence depending on the cell type examined. MiR-34a has also been implicated in both replicative senescence and stress induced premature senescence. Using a real time RT-PCR method we find that miR-34a is expressed at higher levels in fibroblasts from HGPS patients compared to fibroblasts from normal control donors. Transfection of miR-34a also inhibited progeria cell proliferation. Conversely, inhibiting endogenous miR-34a using miRNA inhibitors increased proliferation of progeria cells. Western blot analysis showed that CDK4 and cyclin D1 were downregulated by the transfection of miR-34a, possibly explaining its growth inhibitory effect. Overexpression of miR-34a induced a G1 arrest in progeria cells as estimated by flow cytometry.

These results indicate that miR-34a is expressed at higher levels in HGPS fibroblasts and that it may contribute to inhibiting progeria cell proliferation.

POSTER #10

MicroRNA components of the P33 ING1b tumor suppressor network

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The inhibitor of growth (ING) family of type II tumor suppressors contributes to the neoplastic growth of various tumors with p33ING1b, the most highly expressed isoform in human cells contributing to most recognized functions of ING1. Overexpression of ING1b causes cell cycle arrest at G1 phase with ensuing apoptosis, whereas suppression of its expression increased colony focus formation and growth *in vitro* and tumor formation *in vivo.*

MicroRNAs (miRNAs) are small, non-coding RNA molecules, which regulate gene expression by decreasing target mRNA levels or by directly inhibiting translation. MicroRNA-203 (miR-203) was reported to promote cell differentiation and restrict cell proliferative potential through targeting DeltaNP63, which is an essential regulator of epithelial stem cell maintenance. Like ING1b, miR-203 is also involved in UV-induced apoptosis in squamous cell carcinoma.

We overexpressed ING1b and noted that the expression level of miR-203 increased significantly while inhibiting growth and inducing apoptosis in normal primary Hs-68 cells fibroblasts. U2OS osteosarcoma cells were found to be considerably more prone to ING1b-induced growth inhibition and apoptosis compared to Hs-68 cells and overexpression of miR-203 inhibited U2OS cell proliferation in a dose-dependent manner. We are currently testing whether miR-203 might mediate the tumour-suppressive functions of ING and thus have potential therapeutic value for the future treatment of osteosarcomas and other cancers.

POSTER #11

Protein phosphatase 1 (PP1) is part of a multi-protein, mitotic complex via a direct interaction with the RNA helicase Ddx21

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Metazoan mitosis requires remodelling of sub-cellular structures to ensure proper division of cellular and genetic material. Faults often lead to genomic instability; cell cycle arrests and disease onset (e.g. cancer) or cell death. These key structural changes are under tight post-translational control, with crucial roles for reversible phosphorylation. Mitotic kinases have been researched in-depth, providing a thorough understanding of their actions throughout mitosis. Their counteracting enzymes, the mitotic protein phosphatases, lag behind in both their identification and unravelling their precise impact on mitotic events due to for example the inherently transient nature of the interactions between the subunits that make up each protein phosphatase. Hence, although a number of mitotic PP1 and PP2A complexes have been annotated with essential mitotic roles, others await identification. We present a strategy to enrich and identify phospho-protein phosphatases at the mitotic spindle. Our modular approach makes it generally applicable to other enzyme classes. Moreover, our methodology can isolate low abundance and/or transient phospho-protein phosphatase complexes. We identified a nucleolar RNA helicase, Ddx21, as a novel direct PP1 interactor. Furthermore our results place PP1 within the so-called Toposome, a TOPOIIα-containing complex with a crucial role in mitotic chromatin regulation and cell cycle progression.

POSTER #12

Investigating NHEJ: A biochemical assay to study protein recruitment to DNA double-strand breaks

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DNA integrity is continually challenged by exposure to endogenous and exogenous agents that cause many different types of DNA damage. A major form of damage is the double-strand DNA break (DSB), which if mis- or un-repaired may be mutagenic or cytotoxic. Ionising radiation-induced DSBs, characterized by base lesions, abasic sites or single-strand breaks in close proximity to the DSB termini, are believed to be a major cause of the biological effects of radiation exposure. The type, yield and spatial orientation of IR-induced DSBs is often diverse and thus requires intense co-ordination of repair.

Non-homologous end joining (NHEJ) is the predominant DSB repair pathway in mammalian cells. Key steps of the pathway include recognition/signalling of the break, recruitment of repair factors, processing of the DNA and ultimately ligation of the break. Although, the 'core' NHEJ components are widely characterised, little is known about the order of recruitment, structural conformations adopted during repair, the interplay between different repair pathways (including base excision repair) or the resolution and dissociation of repair factors from the DNA.

Here, we have developed a biochemical assay to study the interaction of repair factors with modeled radiation-induced DSBs. Synthetic oligonucleotides are incubated with extracts from unirradiated or irradiated human cells. Protein recruitment to the breaks is studied in the context of DNA structure and the potential role of phosphorylation on recruitment and repair with the aim of gaining new insights into possible hierarchies of protein recruitment, the role of back-up repair pathways and the interchangeability of repair proteins.

POSTER #13

Mitochondrial defects impair the transcriptional response to removal of glucose

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The genome encoded by mitochondrial DNA (mtDNA) is essential for energy generation via oxidative phosphorylation but yeast and some human cell types do not required mtDNA for viability. The mtDNA is susceptible to damage and loss during replicative aging. During proliferative growth, the differences between the transcriptional profiles of cells without mtDNA (ρ^0 cells) and cells with mtDNA (ρ+ cells) are well characterized. Less is known about the response of ρ^0 cells to variations in nutrient availability. Global transcriptional profiling was employed to compare the response of ρ^+ and ρ^0 cells to removal of glucose from their growth media. During the shift to media without glucose ρ^0 cells displayed abnormal regulation of genes whose transcription is up regulated by the AMPK homologue Snf1, as well as genes which are under the control of TOR and PKA signaling. Further characterization of these pathways demonstrates that activation of Snf1in response to glucose removal occurs with similar kinetics in ρ^+ and ρ^0 cells. However, ρ^0 cells failed to display increased glycogen synthase kinase 3-β mediated phosphorylation of several transcription factors controlled by the TOR and PKA pathways. We employed chemical perturbations and genetic manipulations to examine the mechanism underlying this defect.

POSTER #14

New insights into phosphatase domain of PNKP

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Maintenance of genomic integrity relies on a large number of enzymes and proteins that recognize and repair DNA damage. Among them PNKP (Mammalian Polynucleotide Kinase/Phosphatase) has a significant role in both DNA single strand and double strand break repair by generating the 3'-hydroxyl and 5'-phosphate DNA termini that are required for synthesis of new DNA and ligation of broken DNA ends. The phosphatase activity of PNKP is particularly critical for the repair of damaged DNA generated via ionizing radiation and oxidative processes. However, how the phosphatase domain binds diverse DNA substrates is not understood. Here we show the impact of different site directed PNKP mutants on binding affinities and catalytic activities with model DNA substrates in phosphatase domain. These data help us to understand the mechanism of substrate recognition by PNKP and will also provide a basis for the further development of PNKP inhibitors, which could provide new radio-sensitizing agents.

POSTER #15

Revealing HARP's annealing

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HARP (HepA-related protein) is a protein that is involved in stabilization of stalled replication forks during DNA replication and the overall maintenance of genome integrity (Bansbach et al. 2009; Driscoll & Cimprich 2009). Mutations in the HARP gene are responsible for an autosomal recessive disorder Schimke immunoosseous dysplasia, which can manifest a wide range of effects, such as skeletal dysplasia, kidney failure and T-cell immunodeficiency. The function of HARP, in vivo, remains unknown, however, in vitro, it has been shown to be stimulated by forked DNA structures (Bansbach et al. 2009) and can reanneal single stranded DNA (ssDNA) bubbles of plasmid DNA that has been stably bound by replication protein A (RPA) (Yusufzai & Kadonaga 2008). We propose to determine the structure of HARP, and hypothesize that it will reveal many unknowns surrounding this important protein. We were recently able to establish an expression and purification of a mammalian form of HARP, providing a route for its detailed study. The structure of HARP will provide clues and answers to exciting questions such as: What type of DNA substrate(s) does HARP act on and what sort of 3-dimensional structure facilitates HARP's unique annealing activity (not found in any other protein to-date)?

POSTER #16

Structural determination of the Rad53-binding domain of *Saccharomyces cerevisiae* Dbf4

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The conserved Dbf4/Cdc7 kinase (DDK) is essential for the initiation of DNA replication, and is also a target of the intra-S-phase checkpoint response. During the checkpoint response the DDK regulatory subunit, Dbf4, is phosphorylated in a Rad53 dependent manner leading to inhibition of late origin firing. We have previously shown that the N terminus of Dbf4 is required to mediate the interaction with Rad53, and this region has been predicted to contain a BRCT-like domain. Here, we have determined the minimal region of Dbf4 that is both necessary and sufficient to mediate the interaction with Rad53, and by solving the crystal structure of this region show that it adopts a modified BRCT fold with an additional N terminal α helix (herein referred to as HBRCT (Helix-BRCT) domain). The integrity of this HBRCT fold is paramount to maintain the interaction between Dbf4 and Rad53. We demonstrate that mutations which destabilize the HBRCT fold result in decreased affinity for Rad53 and increased sensitivity to genotoxic stress, indicating that the structural integrity of this domain is important for proper checkpoint response.

POSTER #17

Uncovering mechanisms used during phage T4 DNA replication to bypass DNA damage

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DNA polymerases rarely make mistakes, but high fidelity depends on the ability to avoid replication of damaged DNA. Replication repair, in which the DNA polymerase upon encountering DNA damage switches to a damage-free homologous template, was discovered in bacteriophage T4. We are extending these studies using genetic methods to uncover components of this process and also to determine the role of the T4 DNA polymerase. We have observed that T4 phage that lack recombinase (UvsX) cannot replicate DNA in two different bacterial hosts: (1) *galU* which has low UDP-glucose and (2) *optA1* which has increased dGTPase production. *GalU*-resistant UvsX deficient phage have been isolated. The suppressor mutations are all in the *dda* helicase gene and the mutations are identical in encoding the A392T amino acid substitution. A mutator phenotype is observed for for uvsXam phage as expected if translesion synthesis rather than error-free recombination is taking place. We have a class of mutant T4 DNA polymerases that display the same mutation spectrum implicating reduced recombination.

POSTER #18

The role of prolactin in chemotherapeutic resistance

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High serum levels of the peptide hormone prolactin is associated with increased breast cancer risk. We identified that the isoform Hsp90alpha is a prolactin regulated gene in breast cancer cells. We discovered prolactin induces breast cancer cell resistance to DNA damaging chemotherapeutic drugs and involves the master cancer chaperone heat shock protein-90 (Hsp90). Our hypothesis is that cross-talk between prolactin pathway and DNA damage response is important in prolactin mediated chemoresistance. When breast cancer cells were treated with the Hsp90 inhibitor 17AAG and doxorubicin, a decrease in ataxia- telangiectasia mutated protein (ATM) (a key protein in DNA damage response) and phospho (active) ATM levels was observed, which indicates interaction of Hsp90 with ATM. 17AAG causes proteasomal degradation of Hsp90 client proteins and by using a proteasome inhibitor MG132 with 17AAG, our preliminary data show proteasomal degradation of phosho-ATM. In *in vivo* studies by injecting serial dilutions of breast cancer cells treated with prolactin and doxorubicin, prolactin decreased latency of control and doxorubicin-treated cells. Consequently, current *in vitro* and *in vivo* studies address the mechanism of prolactin mediated cytotoxic resistance and the cross-talk with the DNA damage response.

POSTER #19

Checkpoint adaptation in human cancer cells

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We have developed an experimental model to study checkpoint adaptation in human cancer cells. We treat human cell lines HT29, M059K or U2OS cells with clinically relevant cancer drugs, such as alkylating agents, Topo I inhibitors, Topo II inhibitors or cisplatin. These cytotoxic agents cause distinct types of DNA damage, yet, they induce a reproducible sequence of molecular events including gamma histone H2AX induction, phosphorylation of Chk1 on ser345 and cyclin B1 accumulation. Starting by 48 hours after treatment, cells enter mitosis with their DNA still damaged. Chk1 inactivation by dephosphorylation appears to be closely linked to checkpoint adaptation because changing the timing of entry into mitosis also changes the timing Chk1 dephosphorylation. We have identified small molecules that can either enhance checkpoint adaptation by inhibiting Chk1 activity or block checkpoint adaptation by inhibiting Cdk1 activity. In the case of MO59K cells, a small number of cells survive checkpoint adaptation and display micronuclei, which is a sign of genome rearrangement. These combination treatments enable us to evaluate the relationship between DNA damage, checkpoint adaptation and cytotoxicity. Our data will help improve the outcome of cytotoxic treatments because we will understand better how cells respond to damaged DNA.

POSTER #20

Structural basis for the DNA replication checkpoint interaction between TopBP1 and MDC1

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DNA replication checkpoint control relies on a number of protein-protein interactions that are mediated by the BRCT domains of TopBP1. Recently, an interaction between TopBP1 and MDC1 was identified to be critical for inducing checkpoint activation. This interaction involves the fifth BRCT domain of TopBP1 and the conserved Ser-Asp-Thr (SDT) repeats of MDC1. To gain insight into the mechanism of this interaction, we solved the crystal structures of TopBP1 BRCT4/5 both free and in complex with a consensus MDC1 phospho-peptide. The structure of TopBP1 BRCT4/5 reveals an unusual packing of the BRCT domain interface. In addition, MDC1 phospho-peptide binding involves contacts from two TopBP1 BRCT4/5 protomers, suggesting the requirement for oligomerization of TopBP1 to foster a stable interaction. Taken together, our structural data suggest a novel mechanism for phospho-peptide recognition by BRCT domains.

POSTER #21

Designing a Protein Phosphatase One (PP1) regulatory subunit interaction network In human breast cancer cells

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Breast cancer is the most common invasive cancer in women around the world. Cancer usually involves a mechanistic error in transcription, translation, or protein modification. Most cancer biologists have focused on genotypic mechanisms of cancer development rather than errors in protein regulation such as reversible protein phosphorylation. Biological systems use the counteracting regulatory proteins kinases and phosphatases to control reversible phosphorylation. The Phosphoprotein Phosphatase Family (PPP) dephosphorylates serine and threonine residues and includes Protein Phosphatase One (PP1) which is regulated by hundreds of additional subunits or interacting proteins. The RVS/TF motif is an interaction site for PP1 and its interactors and is known to be phosphorylated in a variety of regulatory subunits. Using affinity chromatography and Mass Spectrometry, new nuclear PP1-interacting proteins have been identified which are known to be phosphorylated at the RVS/TF motif and contribute to breast cancer (ASPP1, Rif1, Ki67, and RRP1B). These PP1 regulatory subunits may interact with other novel proteins which may be regulated by PP1 dephosphorylation or RVS/TF phosphorylation. To characterize this role, a PP1 regulatory subunit interaction network in MCF7 cells will be developed using SILAC mass spectrometry. This proteomic study will help us determine additional binding partners potentially involved in breast cancer development.

POSTER #22

Role of epigenetic effectors in the radiation response of mammary tissue

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Breast cancer is the most common neoplastic disease in the female. 5% of breast cancers are hereditary while 95% are due to environmental mutagens. Ionizing radiation is an energy traveling as waves or particles that can change chemical composition of matter. The main target of ionizing radiation is DNA, which when damaged, can initiate neoplastic transformation of cells. Medical diagnostics and treatment tools are the significant sources of low dose irradiation in human. Therefore, it is important to explore molecular nature of the effects of low (mammography-like) and high (treatment-like) doses of radiation in breast tissue and to correlate these effects with radiation-induced carcinogenesis. Radiation exerts profound global epigenetic DNA methylation and miRNA changes in exposed mammary gland which may be important in breast carcinogenesis. Gene expression analysis showed immediate upregulation of genes involved in natural immune response of rat mammary tissue to radiation treatment. High dose X-ray exposure led to overexpression of lipocalin-2 and cathepsin K oncogenes 24 weeks after treatment. Radiation exposure caused changes in microRNome which are associated with apoptotic response of rat mammary gland. In purpose to evaluate DNA methylation status in IR-exposed mammary tissue, COBRA assay for analysis of LINE-1 was conducted. LINE-1 is a transposable retroelement that constitutes almost 23% of rat genome. 96 hours after radiation treatment LINE-1 promoter was hypomethylated. Such change could result in genome instability, genome rearrangements and possible neoplastic cell transformation.

POSTER #23

The XLF C-terminal region is required for DNA binding and interaction with Ku70/80 *in vitro* but not for repair of double-strand breaks *in vivo*

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DNA double strand breaks (DSBs) are one of the most detrimental DNA lesions in the cell. DSBs can be induced by ionizing radiation (IR) and in mammalian cells these DSBs are primarily repaired by the non-homologous end-joining pathway (NHEJ) which involves several core proteins including the DNA-PK complex which is composed of the Ku70/80 heterodimer and the DNA-PKcs catalytic subunit; the XRCC4-DNA ligase IV complex; and the XRCC4-like factor (XLF) which has been shown to stimulate XRCC4-DNA ligase IV mediated DNA end-joining.

Crystal structures of XLF confirm that it is structurally similar to XRCC4 yet contains noticeable differences in the C-terminal region. Previously, we have shown that the C-terminal region of XLF binds DNA *in vitro* and is phosphorylated by DNA-PK *in vivo*. Here we show that this region, which is predicted to be unstructured, is also required for the DNA-dependent interaction between XLF and the DNA-PK complex in cell extracts and for interaction with purified Ku70/80 in pull-down assays. Interestingly, the highly conserved penultimate amino acid F298 is absolutely required for the interaction between XLF and Ku as well as for DNA binding in electrophoretic mobility shift assays. However, C-terminal truncation of XLF or mutation of F298 does not significantly alter the kinetics of gamma-H2AX foci resolution following IR *in vivo*. These results suggest that although the C-terminal region of XLF is important for DNA binding and interaction with Ku *in vitro,* these interactions may not be necessary for double-strand break repair *in vivo*.

POSTER #24

The exploitation of synthetic lethal relationships with PNKP in targeted cancer therapies

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Synthetic lethality arises when the combination of two non-essential protein disruptions in a cell causes lethality. This phenomenon has been shown to occur between proteins involved in DNA repair and much attention to date has focused on PARP.

We performed an siRNA screen of Qiagen's druggable genome to identify synthetic lethal partnerships with another DNA repair protein, PNKP. The screen was done in duplicate using the A549 stably depleted of PNKP and then again in duplicate using A549 stably expressing a scrambled shRNA.

We have identified several tumor suppressors showing synthetic lethality with PNKP, such as the tyrosine-protein phosphatase SHP-1. SHP-1 expression was shown to be diminished or absent in 40/45 malignant prostate tissues, 95% of malignant lymphomas and 100% of NK and T cell lymphomas. Further investigation has revealed that there is increased ROS production in SHP-1 depleted cells, which provides a possible mechanism for the synthetic lethality seen between PNKP and SHP-1.

Interestingly, since healthy cells can withstand PNKP disruption, only the SHP-1 depleted cancer cells will be affected by treatment. This allows a highly selective, patient specific cancer therapy. Furthermore, since normal tissues can withstand treatment, the side effects typically associated with cancer therapies should be negligible.

POSTER #25

Structure-function analysis of the PNKP-pXRCC1-Ligase 3 interaction in base excision repair (BER)

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All cancers initiate through genetic mutations that arise as a result of damage to DNA. Human cells contain elaborate mechanisms that have evolved to recognize and repair damaged DNA, thereby protecting our genetic information. Our lab studies the proteins that are involved in detecting DNA damage and repairing the damage. This project focuses on the DNA repair enzyme polynucleotide kinase/phosphatase (PNKP), which is a critical enzyme in the repair of broken DNA ends.

Mutations in PNKP make human cells highly sensitive to DNA damage, and also are associated with a form of a developmental neurological disorder called MCSZ. This research aims to understand how PNKP recognizes and repairs broken DNA ends, and how it interacts with other proteins to help in the DNA repair process, X-ray cross complementing protein 1 (XRCC1) and ligase 3 (Lig3). Determination of the crystal structure of the PNKP complex with XRCC1 and Lig3 contributes to our knowledge of the mechanism of repair damaged DNA in the cell. This work will provide insights that will help us to develop molecules that inhibit PNKP function that could provide leads in the development of new cancer therapies. Determination of the 3D structure of the PNKP complex with inhibitors will enable us to characterize the structural aspects of the inhibitors affinities as well as their binding modes. This work will also provide insights into why specific mutations in PNKP are associated with MCSZ. The specific targeting of DNA repair pathways is an important new direction in cancer therapy development.

POSTER #26

Study of transgenerational methylation status in Arabidopsis in response to hot, cold and UV stress

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Exposing plants to abiotic stress may increase stress tolerance in their progeny, a process probably regulated by epigenetics. DNA methylation, an epigenetic regulatory mechanism, may have a negative correlation with the rate of double strand break DNA repair process homologous recombination because it makes chromatin less accessible to remodeling, increasing genome stability. Small interfering RNAs can maintain DNA methylation and their synthesis relies on Dicer activities, encoded by *dcl2-dcl4* in *Arabidopsis thaliana*. Abiotic factors may destabilize the plant genome through hypomethylation of DNA at stress-related loci. I will analyze epigenetic changes in the progeny of *Arabidopsis* plants exposed to hot, cold and UV stress. The progeny of stressed and non-stressed (control) wild-type and *dcl2*, *dcl3* and *dcl4* mutants will be germinated in normal and stress conditions and physiological parameters will be analyzed to detect stress tolerance. Tissues will be collected for mRNA/miRNA and DNA methylation analyses. The methylation status of stressed and non-stressed (wt and mutants) progeny will be compared to determine the impact of abiotic stress on genome stability transgenerationally. This research is important to help determine if passing epigenetic memory of the abiotic stress exposure to progeny may regulate changes in the genome stability of plants.

POSTER #27

Do stress-induced premature senescence and mitotic catastrophe reflect irreversible growth arrest?

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Cryptogenic γ-H2AX nuclear foci accumulate in senescing human cells and are thought to signify irreparable DNA double-strand breaks. We have reported that early passage p53 wild-type human fibroblasts respond to ionizing radiation by exhibiting persistent γ-H2AX foci, proliferation block, and sustained nuclear accumulation of p21 (but not p16) when measured late times (e.g., 1 week) post-irradiation. Moreover, p53 mutated Li-Fraumeni syndrome (LFS) fibroblasts exhibit replicative senescence coupled with massive cryptogenic γ-H2AX at late passages; early passage LFS fibroblasts respond to ionizing radiation by undergoing stress-induced premature senescence (SIPS) that is associated with extensive genomic instability (e.g., multinucleation), persistent γ-H2AX foci, proliferation block, and induction of p16 (but not p21). We have now extended these studies to solid tumor-derived cells with differing p53 status. We show that exposure to clinically-relevant doses of ionizing radiation triggers the development of “giant” cells, which predominantly reflect SIPS and multinucleation in p53 wild-type and p53-null cultures, respectively. Such giant cells exhibit full clearance of γ-H2AX foci, do not express p21 or p16, and resume proliferation at late times post-irradiation. Thus, radiation-induced growth arrest that is coupled with SIPS and multinucleation (“mitotic catastrophe”) might be permanent in non-cancerous cells but reversible in tumor cells.

POSTER #28

The role of INhibitor of Growth 3 (ING3) in prostate cancer

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Prostate Cancer (PC) is the most common cancer among men worldwide and it is very highly age-related, with incidence increasing dramatically from the 5th decade of life onwards. Although localized PC initially responds well to hormone therapy, the majority of patients ultimately enter a hormone-resistant stage, which remains fairly incurable. The INhibitors of Growth are type two tumor suppressors, which are deregulated in numerous types of cancer. Recent translational studies on melanoma, head and neck carcinoma as well as ameloblastoma have indicated that knowing the status of ING3 levels can have prognostic value. Our preliminary studies on prostate cancer cell lines and patient samples show that high levels of ING3 are associated with more aggressive prostate cancer. *Thus, we hypothesize that ING3 might promote prostate cancer progression through growth stimulatory effects and knowing it's status might be useful for predicting stage and treatment outcome.* To initially test this idea we are examining the effects of knocking down and overexpressing ING3 in these cell lines, with the aim of understanding the underlying mechanisms of ING3 hypothesized growth promotion in PC. Moreover, by doing tissue microarrays, we are planning to determine the levels of ING3 in samples derived from patients with different stages of the disease. By doing these experiments, we hope to find novel prognostic applications of ING3 as a biomarker in prostate cancer.

POSTER #29

The spindle assembly factor TPX2 regulates DNA damage response via MDC1 / ATM-dependent amplification of p-H2AX

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The microtubule-associated protein TPX2 is required for spindle formation and mitosis. In interphase, TPX2 is imported to the nucleus to prevent its premature microtubule organization activity. No function has been assigned to nuclear TPX2. We now report that TPX2 is involved in the response to DNA double strand breaks (DSB) induced by ionizing radiation (IR). TPX2 interacts with MDC1 to regulate the MDC1/ATM-dependent amplification of p-H2AX. Loss of TPX2 leads to inordinately strong p-H2AX accumulation in G0 and G1 and the increased formation of high intensity p-H2AX foci. Pharmacologic inhibition or depletion of ATM, but not of DNA-PK, rescues this phenotype. Moreover, cells lacking TPX2 have defects in recruitment and disengagement of 53BP1 and RAD51, respectively at the sites of lesions, accumulate more DSBs and ultimately, undergo increased apoptosis. Conversely, cells overexpressing TPX2 have reduced levels of p-H2AX after IR. Critically, the regulation of p-H2AX signals by TPX2 is independent from TPX2's mitotic roles as confirmed by gain and loss of function experiments in post-mitotic neurons that have permanently exited the cell cycle. In conclusion, our study unravels a novel and the first nuclear function for TPX2 in the cellular responses to DNA damage.

POSTER #30

Is ING1 function regulated by sumoylation?

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More than a decade has passed since the discovery of the INhibitor of Growth family of proteins (ING1-5 family) and since then over time their importance in cancer progression, DNA repair, senescence, cell cycle, chromatin remodeling and a variety of other cellular processes has been established. Phylogenetically ING1/ING2 and ING4/5 are closely related and ING3 stands distinct. ING1, the first member of this family has 4 known isoforms, ING1a-d. Overexpression of ING1a induces senescence and ING1b overexpression induces apoptosis. ING1/2 and ING3/4/5 as stoichiometric members of HDAC and HAT complexes respectively and regulate a variety of genes. However how ING1-5 are regulated themselves is not well understood. A recent report indicated the addition of small ubiquitin like modifier protein 1 (SUMO-1) to ING2 regulates its association with the Sin3A HDAC complex. The SUMO family of proteins was first discovered in 1997 and structural and biochemical studies showed that like ubiquitin, SUMO is covalently attached through a similar enzymatic cascade, activation, conjugation and ligation as that of ubiquitin. Sumoylation, unlike ubiquitination, does not target proteins for degradation but alters protein stability, localization, transcriptional activation and. The putative consensus SUMO acceptor site within a protein is ΨKXE (Ψ is an aliphatic branched amino acid and X is any amino acid). Interestingly, ING1b contains a putative sumoylation consensus site and we hypothesize that similar to ING2 sumoylation, ING1b is sumoylated and this sumoylation of ING1b can regulate its function as an inducer of apoptosis or its role in chromatin remodeling. We are currently beginning to explore the potential and for possible biological consequences of ING1b sumoylation using site directed mutagenesis of a key lysine in the putative SUMO acceptor site.

POSTER #31

Post replication repair is not always beneficial

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DNA damage blocks DNA replication, but post replication repair (PRR) provides two tolerance mechanisms that allow chromosome replication to be completed. Translesion synthesis (TLS) is an error-prone mechanism that required mono ubiquitylation of PCNA catalyzed by Rad6/Rad18 and the DNA polζ and η TLS DNA pols. Further ubiquitylation of PCNA by Mms2/Ubc13/Rad5 allows error-free recombination to engage. We asked if PRR is needed if DNA replication is hindered without DNA damage. We answered this question by using the L612M-DNA polδ, which is sensitive to the antiviral drug phosphonoacetic acid (PAA). Because DNA polδ is the lagging strand DNA pol, PAA is predicted to inhibit lagging strand replication specifically. We find that deletion of *RAD18* or *REV3*, which encoded the catalytic subunit of DNA polζ, does not affect the PAA-sensitivity of *pol3-L612M* cells, but deletion of *MMS2* or *UBC13* decreases PAA-sensitivity, which suggests that some aspect of PRR recombination mechanisms is deleterious. This proposal is supported by the observation that deletion of *SRS2*, which encodes a helicase that can unwind joint molecules and dissociate the Rad51 recombinase from nucleofilaments, causes severe PAA-sensitivity in *pol3-L612M* cells. We conclude that PRR mechanisms that involve HR are not beneficial; a model will be presented.

POSTER #32

Comparison of radiation sensitivity in aging plant and human cells

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Radiation sensitivity is age-dependent in humans, with children being more sensitive than adults. Stress-sensitivity in plants also seems to depend on developmental stage of the plants. Further, plants tolerate higher doses of ionizing radiation than mammalian systems, which makes a comparison of radiation response in a plant and a mammalian system worthwhile. The current study is aimed at uncovering the molecular mechanisms behind these events.

We employ two cell culture models that "age" *in vitro*. One is the human lung fibroblast WI38 strain that is commonly used in aging studies and has a finite lifetime in culture. The second one is a cell suspension culture of *Arabidopsis thaliana* cells, as plant cell suspension cultures have previously been shown to upregulate senescence-related genes during later stages of their growth cycle and therefore can also mimick a senescence-state *in vitro*.

These two models are used to compare the effects of X-irradiation on global gene expression levels and epigenetic responses, such as methylation levels, histone modifications and small RNA expression profiles. Furthermore, we will compare the capacity of both models to deal with DNA damage, by assessing the accumulation of damage upon irradiation and the capacity of DNA repair machinery to deal with it.

POSTER #33

Epigenetic alterations in the liver of male and female mice primed with a low X-ray irradiation dose

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Ionizing radiation (IR) is an important diagnostic and treatment modality, yet it is also a potent genotoxic agent that causes genome instability and carcinogenesis. It is believed that a low dose exposure to ionizing radiations such as X-rays can help an animal to cope with a later subsequent higher irradiation dose. This effect is termed as “the priming effect”. The role of priming on IR-induced genomic instability is not known. We hypothesized that priming the animals with a low X-Ray dose followed by a challenged higher dose would alter the epigenetic mechanisms in treated animals. To test this hypothesis we utilized an established *in vivo* mouse model to study these mechanisms in the IR-target organ liver. Male and female mice were irradiated with different irradiation doses of (a) challenge dose, (b) primed dose, (c) primed dose + challenge dose or (d) untreated controls. We observed a decrease in the global DNA methylation in primed + challenge dose group compared to challenge and control animals. We also observed a decrease in the hypermethylation pattern of genes implicated in liver cancer. The miRNA profile and expression of miRNA processing machinery was also significantly altered. These findings support our hypothesis that priming alters the gene expression pattern via altering the epigenetic mechanisms.

The study was supported by the DOE and CIHR.

POSTER #34

Radiation-induced bystander effects alter molecular mechanisms *in vivo*

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The bystander effect is a phenomenon whereby directly irradiated cells communicate damage to distant non-irradiated bystander cells resulting in genetic instability. Although *In vivo* data is limited, recent evidence suggests that bystander effects are sex-specific and epigenetically mediated via non-coding RNAs, specifically, microRNAs. miRNAs mediate translational suppression of target mRNA molecules.

The current study investigated the nature and magnitude of radiation-induced bystander effects in a sex-specific Long Evans rat model. To analyze the sex-specificity and persistence of the bystander response, male and female rats were exposed to whole-body and cranial irradiation, with unexposed rats serving as controls. The animals were sacrificed 4 and 14 days after irradiation. Here we report that radiation exposure triggered a significant and sex-specific deregulation of the microRNAome in the directly exposed and non-exposed bystander liver tissue.

Altered miRNAome levels were paralleled by sex-specific changes in the levels of the miRNA processing machinery components and miRNA target proteins. For example, the observed increase in the level of miR-25 in the bystander tissues was paralleled by the respective decrease of its target protein, Bim. Furthermore, altered molecular expression levels were observed in apoptosis and DNA repair machinery in bystander liver tissue of males and females.

POSTER #35

PNKP is required for the repair of mitochondrial DNA damage

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Human polynucleotide kinase phosphatase (hPNK) possesses both DNA 5'-kinase and 3'-phosphatase activities. hPNK contains a forkhead-associated (FHA) domain, and two independent kinase and phosphatase domains. The functional domains are responsible for the generation of the repair-competent DNA ends (5'-phosphate and 3'-OH) following DNA damage.

Here we demonstrate that functionally active full-length PNKP is present in mitochondria. Down-regulation of PNKP results in a decrease of both mitochondrial and nuclear PNKP and accumulation of DNA damage in mtDNA. Furthermore, our results indicate that PNKP associates with mitochondrial proteins mitofilin and Pol Gamma. In addition, we demonstrate that PNKP contains a C-terminal Mitochondrial Targeting Signal. This C-terminal MTS is functional and is required for the localization of PNKP to mitochondria. Unlike amino-terminal MTS that is cleaved upon entry to mitochondria, C-terminal MTS at least in the case of PNKP is not cleaved.

POSTER #36

The MRX complex regulates cohesin during replication: In times of stress keep your sister close

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The Mre11-Rad50-Xrs2 (MRX) complex is important for the maintenance of genomic integrity and is recruited to forks during pauses in replication. One essential function of the MRX complex is to provide architectural support to DNA during repair, meiotic recombination and telomere maintenance. Structural analysis of the MRX complex suggests that it can bridge two strands of duplexed DNA and serve as a long-range tether between sister chromatids. Moreover, a dramatic loss of cell viability in the presence of the DNA replication inhibitor HU is observed when disruptions in MRX are combined with mutations in factors involved in sister chromatid cohesion (SCC). Establishment of SCC is coupled to replication fork progression and is facilitated by the cohesin complex which holds replicated chromosomes together from their synthesis until the onset of anaphase. Using ChIP-Chip analysis we show increased cohesin association with forks when they stall, above levels detected in an unperturbed S phase, and this is dependent on the MRX complex. In addition, we find the MRX complex is necessary for proper sister chromatid cohesion (SCC), and this function depends on the structural integrity and bridging capacity of Rad50. Taken together, our data suggests one critical role for the MRX complex in preserving chromosome integrity involves supporting the architecture of newly synthesized daughter strands during pauses in replication, preventing fork-associated damage.

POSTER #37

The role of long non-coding RNAs in ING1b-induced apoptosis

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The INhibitor of Growth 1 (ING1) is a member of five conserved ING tumour suppressor genes. ING1b is the dominant splicing isoform of the ING1 gene and it, in particular, has significant functions in apoptosis. ING1b can also bind to chromatin at trimethylated histone H3 lysine 4 (H3K4me3) and subsequently recruits chromatin-modifying complexes to alter gene expression. Given the involvement of ING1b in epigenetics, I have asked if it could also function through long intervening non-coding RNAs (lincRNAs), which represent a novel epigenetic mechanism. We suspect that since ING1b binds to chromatin at H3K4me3 marks, which are also indicative of lincRNA promoters, it is possible that ING1b is regulating lincRNA expression as well. LincRNAs would then act as effectors in ING1b-induced processes. Under pathological conditions, such as in cancer, ING1b levels are deregulated. Thus, to further elucidate the role of ING1b in apoptosis, we looked at lincRNA gene transcription after ING1b overexpression and found that its level is greatly increased. This lincRNA has been shown to act by repressing genes that normally inhibit programed cell death. Therefore, we *hypothesize* that lincRNA-p21 is also modulating gene repression downstream of ING1b and thereby constitutes a critical link in transducing ING1b-induced apoptosis.

POSTER #38

Bacteria determinants on genome stability of direct and distal cells

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A significant number of carcinomas identified in the current human population are the result of a previous infection. Bacterial infections can be sourced back to a number of possible areas of contamination such as water contamination. Upon such contamination in water sources, procedures such as Boil Water Advisory have been issued to ensure the water is safe to consume. Despite this advice, the evidence exists that consumption of heat-killed bacteria and lipopolysaccharides (a component of the bacterial membrane) leads to the genomic instability of cells in direct contact of the water (intestinal cells) as well as distal cells (liver and spleen cells). We hypothesize that exposure to a pathogen's components would result in a decrease in genomic stability and an increase in the rearrangement in other areas of the genome. Mice models separated into groups each exposed to bacterial determinants (DNA, RNA protein or LPS) or to whole heat-killed bacteria for a short period of time were established to test this hypothesis. The results present the molecular consequences that heat-killed bacterial components induce genome instability of cells exposed directly or indirectly based on the "bystander effect" theory. Exposure to these determinants results in alteration of mRNA and expression, proliferation and methylation. Exposure to determinants from the bacterium *E. coli* O157:H7 results in significant alterations within intestine, liver and spleen cells of the mice models.

POSTER #39

Homologous recombination repair is required for the resolution of estrogen-induced DNA double strand breaks

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Previously, we have shown that estrogen induces the formation of DNA DSBs through a mechanism dependent on ERα-dependent transcription. Estrogen-induced DSBs occurred specifically in S and G2 phases of the cell cycle and DNA replication was implicated in the mechanism of DSBs formation. Here we examined the role of HRR in the repair of estrogen-induced breaks. We show exposure of cells deficient in HRR by treatment with the mre11 inhibitor, mirin, or depletion of Rad51 to estrogen results in persistent γH2AX foci which is indicative of a defect in the repair of estrogen-induced DSBs. Accordingly, a deficiency in HRR inhibited estrogen stimulated proliferation and increased the level of spontaneous cell death consistent with an accumulation of un-repaired DSBs. Furthermore, exposure of mirin treated and Rad51 depleted cells to estrogen resulted in an accumulation of cells in G2 which suggests the presence of un-repaired DSBs inhibits the progression of G2 cells into mitosis. We propose the HRR pathway is critical for the repair of estrogen induced DSBs.

POSTER #40

The association of Jumonji JMJD2 proteins with DNA damage response-induced chromatin remodelling

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One aspect of the DNA damage response (DDR) is chromatin remodelling through specific post-translational histone modifications. Changes in ubiquitylation, phosphorylation, acetylation and methylation alter chromatin structure, permitting access of DNA repair machinery, creating sites for repair and signalling proteins, and repressing transcription of neighbouring genes. The JMJD2 subfamily of Jumonji demethylases contribute to the regulation of cellular differentiation and proliferation; mutation or aberrant expression of these proteins has been identified in human tumours. These four proteins (JMJD2a-d) have a N-terminal catalytic core and remove trimethylation at histone lysine 9, thus removing the heterochromatin protein 1 (HP1) binding site which is expected to loosen chromatin organization. We hypothesize that recruitment of JMJD2 family members to DNA damage sites is required for the decondensation of heterochromatin in response to DSBs, and hence contributes to the progression of DSB repair. Using laser micro-irradiation, we found that JMJD2b is recruited to DNA damage and that recruitment was ATM-independent, but was partially dependent on poly (ADP-ribose) polymerase 1 activity. Initial experiments revealed that preservation of the catalytic core was required for JMJD2b recruitment. Experiments will be expanded to fully delineate the domain requirement for JMJD2B recruitment to DNA damage, and to include the remaining JMJD2 members.

POSTER #41

Role of translesion synthesis DNA polymerases in DNA replication in the presence of a mutant DNA polymerase δ in *Saccharomyces cerevisiae*

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We have isolated DNA pol δ mutants in yeast that are sensitive to hydroxyurea (HU). Because the mutant DNA pols need higher concentrations of dNTPs than the wild type DNA pol δ, we predict that replication is impaired even when dNTP pools are at normal concentrations. Researchers (1,2) propose that reduced replication capacity of DNA pol δ mutants provides increased opportunity for translesion synthesis (TLS) DNA pols to participate in chromosome replication. We have tested this proposal with the HU-sensitive V592G-DNA pol δ in the presence of mismatch repair (MMR) as done by others, and in the absence because DNA replication at replication forks is subject to MMR. In the presence of MMR, there is a striking increase in GC to CG mutations as reported, which supports a role of DNA pol ζ, but these mutations are not observed in the absence of MMR. Instead, GC to AT, GC to TA, and TA to AT mutations are increased, which are due to TLS DNA pols. We propose that increased TLS in *pol3-V592G* cells is because the mutant DNA pol δ has reduced ability to carry out error-free recombination at sites of damage.

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(2) Mito *et al*. (2008) *Genetics* 179: 1795.

POSTER #42

Prenatal stress modulates epigenetic regulation to determine brain function across the lifespan

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Introduction: Studies have shown that prenatal stress affects health across the lifespan. Epigenetic modifications, such as microRNAs (miRNAs) have been implicated with gene expression regulation and translation inhibition. Here we provide an epigenetic link between adverse experience, gene expression and behavioural disturbances in adulthood.

Hypothesis: Prenatal stress influences miRNA and mRNA expression in newborn animals. Such modulation is related to brain pathology and behavioural disturbances in adulthood.

Methods: Newborns prenatally stressed and their non-stress controls were sacrificed immediately after birth for analysis of the microRNAome and transcriptome using Illumina microarrays (n=3 per group). Two additional groups of prenatally stressed rats were raised to investigate the effects of prenatal stress on behavioural outcome and stress response in adulthood (n=16). The adult rats were tested in open field and ladder rung walking tasks on postnatal day 90, and after 1 week of stress regimen. Non-prenatally stressed rats were used as controls (n=8).

Results: Prenatal stress modulated the expression of several brain miRNAs ($p<0.05$) targeting genes related to neurotrophic factors, neurotransmission, stress response, cell signaling, metabolism, and genes involved with miRNA biogenesis, such as Dicer1. The alterations in miRNA and mRNA expression were accompanied by disturbed skilled limb use and altered open field exploration indicative of elevated anxiety in prenatally stressed animals.

Conclusion: Prenatal adverse experience affects the transcription of brain miRNAs related to central physiological pathways. Additionally, our findings suggest disturbance in locomotion and anxiety related to prenatal stress or cumulative stressful experiences. These findings show that prenatal stress leads to changes in the epigenome, and consequently in the transcriptome, that may cause altered behavioural profiles.

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