Robson DNA Science Centre

10 Year Anniversary Symposium

Friday, November 22, 2024

Robson DNA Science Centre 10-Year Anniversary Symposium on the Biology of DNA Repair and Cellular Aging.

Alt Hotel – University District
Friday, November 22, 2024, from 1:00 – 7:00 pm.

We are thrilled to have the following speakers join us:

- Dr. Peter Stirling, Professor, University of British Columbia
- Dr. Haley Wyatt, Associate Professor, University of Toronto
- Dr. Amélie Fradet-Turcotte, Professor, Laval University
- Dr. Rémi Buisson, Associate Professor, University of California, Irvine

AGENDA

1:00 PM	**Welcome (Indigo Room)**
1:05 – 2:50 PM	**Session chaired by Dr. Aaron Goodarzi**
1:05 – 1:30 PM	Dr. Amélie Fradet-Turcotte, *A chromatin path to DNA repair*
1:35 – 1:50 PM	Dr. Aaron Goodarzi, *CHD chromatin remodelling in the DNA damage response as an emerging anti-cancer target.*
1:50 – 2:20 PM	Dr. Peter Stirling, *Genetic mapping of translesion synthesis: Two ways*
2:20 – 2:35 PM	Dr. Dave Schriemer, *4D proteomics: context matters!*
2:35 – 2:50 PM	Dr. Jennifer Cobb, *Aging, DNA damage processing, and Genome Instability*
2:50 – 3:10 PM	**Refreshment Break (Foyer)**
3:10 – 5:10 PM	**Session Chaired by Dr. Pierre Billon**
3:10 – 3:40 PM	Dr. Rémi Buisson, *Mechanism of Fork Breakage during Replication Catastrophe*
3:40 – 3:55 PM	Dr. Pierre Billon, *From improved Delivery to Detection: Molecular Tools for Efficient CRISPR-based Genome Editing*
3:55 – 4:10 PM	Dr. Karl Riabowol and Dr. Tara Beattie, *"Extending Cell Lifespan"*
4:10 – 4:40 PM	Dr. Haley Wyatt, *Phosphorylation of SLX4 drives folding of the SAP domain and interaction with MUS81-EME1*
4:40 – 4:55 PM	Dr. Gareth Williams, *How structure d*rives function in DNA damage response chromatin *remodellers*
4:55 – 5:10 PM	Dr. Susan Lees-Miller, *Adventures in DNA double strand break repair*
5:10 PM	**Closing Remarks Dr. Susan Lees-Miller and Dr. Aaron Goodarzi**
5:15 – 7:00 PM	**RECEPTION and TRAINEE POSTER SESSION (Foyer)** *Hot Canapes and Charcuterie Served alongside Cash Bar*

We look forward to celebrating the 10th Anniversary of the Robson DNA Science Centre with you.

Arnie Charbonneau Cancer Institute
Robson DNA Science Centre
UNIVERSITY OF CALGARY
Alberta Health Services

SPEAKER ABSTRACTS

Amelie Fradet-Turcotte

Title: A chromatin path to DNA repair

DNA damage signaling and repair are critical processes that prevent the accumulation of genomic alterations that drive carcinogenesis in mammalian cells. These processes are tightly regulated, with the recruitment of DNA repair factors depending on a series of post-translational modifications on chromatin surrounding the damage site. At DNA double-strand breaks, histone H2A lysine 15 (H2AK15) is ubiquitinated by the E3-ubiquitin ligase RNF168, creating a docking site for various DNA repair factors, including 53BP1, BARD1, RNF168, RNF169, and RAD18. Although these factors all recognize the same histone mark, the specificity of their recruitment is essential for ensuring the correct DNA repair pathway is chosen between non-homologous end-joining (NHEJ) and homology-directed repair (HDR). In this presentation, I will discuss our current understanding of how the chromatin environment at DNA breaks influences the choice of repair pathway, and how this choice can extend beyond double-strand break repair to affect broader aspects of genome stability.

Aaron Goodarzi

Title: CHD chromatin remodelling in the DNA damage response as an emerging anti-cancer target

To tolerate genotoxic stress and preserve health, cells enable DNA repair responses where adjustment of chromatin compaction (near a DNA lesion) is often needed for repair to occur. Amongst the many chromatin remodellers understood to be involved in DNA damage response, a majority of those in the CHD family of enzymes have clear roles in genomic stability, human health, DNA repair and/or cancer evolution, including CHD1, CHD2, CHD3.1, CHD4, CHD5, CHD6, and CHD7. Many of these enzymes are recruited to DNA damage via processes regulated by poly(ADP-ribose) (PAR) polymerase 1 and 2 (PARP-1/2) enzymes – whose inhibition and trapping on DNA is considered one of the most effective interventions against cancers lacking BRCA1/2-pathways of DNA damage repair. Over the past decade, our team has demonstrated that the CHD6 chromatin remodeler responds to oxidative stress associated DNA damage, and that mutating CHD6 sensitizes cells to PARP-1/2 inhibitors in a manner distinct from BRCA1.

While CHD6 loss does not impair RAD51 foci formation or DNA double-strand break repair, it causes sensitivity to multiple causes of replication stress, PARP-1/2-trapping or Pol ζ inhibitor-induced γH2AX foci accumulation in S-phase, and the hyper-accumulation of PARP-1 bound or retained to oxidatively-damaged chromatin. DNA repair pathway screening reveals that CHD6 loss elicits insufficiency in apurinic-apyrimidinic endonuclease (APEX1) activity and genomic abasic site accumulation, while interactome analysis finds a majority of proteins associated with CHD6 are either PAR-associated or PAR-modified. We have revealed a newfound, APEX1-linked role for CHD6 in the DNA damage response that is important for understanding PARP-1/2-trapping inhibitor sensitivity and, potentially, represents a novel route towards anti-cancer intervention.

Peter Sterling

Title: Genetic mapping of translesion synthesis: Two ways

DNA damage tolerance pathways recognize stalled DNA replication forks late in S-phase and act to help cells complete DNA replication through two pathways. Template-switching is a recombination based pathway that uses the sister chromatid to bypass the lesion, while translesion synthesis (TLS) uses specialized DNA polymerases that use permissive active sites to accomodate and directly bypass damaged DNA bases. TLS polymerases have been suggested as therapeutic targets in cancer where they often act to maintain tumour cell viability and support chemoresistance. Indeed, synthetic lethal strategies have been proposed (e.g. between POLZeta and Homologous-recombination deficiency), although the genetic network of TLS polymerases is incompletely understood. In this presentation, I will present two orthogonal genetic approaches that interrogate the genetic contexts in which TLS inhibition might be useful, and explore the structure-function relationships within individual TLS polymerases that might be targetable by small molecules. In part 1 I will describe an isogenic knockout collection we have created for the four Y-family polymerases in human cells and initial CRISPR-based synthetic lethal screening. In part 2 I will describe deep mutational scanning for dominant negative forms of DNA polymerase eta using a yeast model for genotoxicity. This analysis identifies PCNA-dependent and allosteric mutation sites on POL-eta that might provide optimal sites for small molecule development. We hope that integrating these types of approaches will reveal new TLS polymerase biology that can be deployed in the search for new anti-cancer therapeutics targeting DNA repair.

Dave Schriemer

Title: 4D proteomics: context matters!

Proteomics is known for cataloging and quantifying proteins in complex samples, but it needs to be far more than a bulk profiling technology. To truly understand cellular behavior, we need to map the higher-order properties of the proteome as well. These properties are fundamentally organizational and temporal. Where are the proteins, and what are they doing? Spatiotemporal behavior is derived from the structure and interactions of proteins but we don't have a technology that can track these characteristics at the proteome level. A few years ago we set out to reimagine proteomics as a technology with high spatial fidelity. In this talk we will introduce our program in 4D proteomics that is based upon intracellular crosslinking and describe how it could be used to address questions in DNA damage repair.

Jennifer Cobb

Title: Aging, DNA damage processing, and Genome Instability

Age is the number one predisposing factor for receiving a cancer diagnosis. Older cells and cancer cells have many similar features, such as increased DNA damage and genome instability. Old cells use alternative mutagenic pathways more frequently to repair DNA damage. By understanding the relationship between age and cancer initiation at the molecular level we hope to identify novel therapeutic targets. The overarching goal is to uncover new ways by which the DNA damage response in old cells can be leveraged for therapeutic benefit in cancer

Rémi Buisson

Title: Mechanism of Fork Breakage during Replication Catastrophe

ATR is the master safeguard of genomic integrity during DNA replication. Acute inhibition of ATR with ATR inhibitor (ATRi) triggers a surge in origin firing, leading to increased levels of single-stranded DNA (ssDNA) that rapidly deplete all available RPA. This leaves ssDNA unprotected and susceptible to breakage, a phenomenon known as replication catastrophe. However, the mechanism by which unprotected ssDNA breaks remains unclear. Here, we reveal that APOBEC3B is the key enzyme targeting unprotected ssDNA at replication forks, triggering a reaction cascade that induces fork collapse and PARP1 hyperactivation.

Pierre Billon

Title: From improved Delivery to Detection: Molecular Tools for Efficient CRISPR-based Genome Editing

CRISPR-based genome editing technologies are rapidly advancing basic research and clinical applications, with remarkable recent successes in treating genetic diseases and cancer. The advent of new tools, like base editing and prime editing, has expanded the scope and precision of genetic modifications, from single nucleotide changes to gene-sized integrations. However, achieving high editing efficiency, and accurately detecting desired genetic changes remains critical obstacles for their widespread adoption. In this presentation, I will highlight our recent work in addressing these challenges.

In particular, we have developed a novel methodology that uses enveloped nanoparticles to deliver genome editing ribonucleoproteins into mammalian cells. By combining the high infectivity of viral-like particles with the robust editing capabilities of ribonucleoproteins, we achieve enhanced genome editing efficiencies across various cell types. We demonstrate that this approach can overcome the limitations of traditional lentiviral-based methods in high-throughput CRISPR screens and enable high-efficiency prime editing screens.

Additionally, we have designed a rapid and efficient method for capturing mutations of interest. This method only requires a PCR product to be incubated in an all-in-one reaction for 10 minutes at room temperature. We have devised multiple detection modalities for quantitative, qualitative, and visual detection, and established a rapid clinical diagnostic platform for sickle cell disease that has demonstrated 100% accuracy in identifying pathogenic variants from patients' blood spots and saliva samples. This cost-effective method enriches the toolkit of detection methods for CRISPR-based precision genome editing and clinical applications.

The development of user-friendly molecular tools will ultimately promote the broader accessibility and democratization of genome editing technologies, expediting the realization of their transformative potential.

Karl Riabowol and Tara Beattie
Title: "Extending Cell Lifespan"

Replicative senescence occurs in response to shortened telomeres and is triggered by an ATM and TP53-mediated DNA damage signal that blocks replication. It is widely believed that hTERT lengthens telomeres to block damage signaling and the onset of senescence. We find that normal diploid fibroblasts expressing hTERT mutants that are unable to maintain telomere length do not initiate DNA damage signaling and continue to replicate, despite having telomeres shorter than senescent cells. The TRF1 and TRF2 DNA binding proteins of the shelterin complex stabilize telomeres and we find that expression of different mutant hTERT proteins inhibits expression of the Siah1 E3 ubiquitin ligase that targets TRF2 to the proteosome. This restores the TRF2:TRF1 ratio to block the activation of ATM and subsequent activation of TP53 that is usually associated with DNA damage-induced senescence signaling. All hTERT variants reduce DNA damage signaling and this occurs concomitantly with telomeres assuming a more compact, denser conformation than senescent cells as measured by super resolution microscopy. This indicates that hTERT variants induce TRF2-mediated telomere compaction that is independent of telomere length, and it plays a dominant role in regulating the DNA damage signalling that induces senescence and blocks replication of human fibroblasts. These observations support the idea that the short telomeres often seen in cancer cells may fail to induce senescence due to selective stabilization of components of the shelterin complex, increasing telomere density, rather than the reverse transcriptase activity of hTERT.

Hayley Watt
Title: Phosphorylation of SLX4 drives folding of the SAP domain and interaction with MUS81-EME1

The DNA repair scaffold SLX4 has multifaceted roles in genome stability, many of which depend on structure-selective endonucleases. SLX4 coordinates the cell cycle-regulated assembly of SLX1, MUS81-EME1, and XPF-ERCC1 into a tri-nuclease complex called SMX. Mechanistically, how the mitotic kinase CDK1 regulates the interaction between SLX4 and MUS81-EME1 remains unclear. Here, we show that CDK1-cyclin B phosphorylates SLX4 residues T1544, T1561, and T1571 in the MUS81-binding region (SLX4MBR). Phosphorylated SLX4MBR relaxes the substrate specificity of MUS81-EME1 and stimulates cleavage of replication and recombination structures, providing a biochemical explanation for the chromosome pulverization that occurs when SLX4 binds MUS81 in S-phase.

Remarkably, phosphorylation of SLX4MBR drives folding of a SAP domain, which underpins the high-affinity interaction with MUS81. We also report the structure of phosphorylated SLX4MBR and identify the MUS81-binding interface. Our work provides mechanistic insights into how cell cycle-regulated phosphorylation of SLX4 drives the recruitment and activation of MUS81-EME1.

Gareth Williams

Title: How structure drives function in DNA damage response chromatin remodellers.

During my postdoctoral research with Dr. John Tainer at Lawrence Berkeley National Laboratory, I developed expertise in the structural biology of ATPase complexes, focusing on the homologous recombination repair proteins MRE11-RAD50 and RAD51 family members. In 2016, I joined the University of Calgary, supported by Robson DNA Science Centre (RDSC) funds, to establish my independent lab. This support, for which I will always be grateful, enabled my new lab to advance research into RAD51 and its paralogs while establishing new collaborative projects on the DNA damage response using structural biology approaches.

As we celebrate the 10-year anniversary of the transformative Robson gift, I reflect on the research accomplishments it made possible. Among these, I am most proud of the structural and mechanistic insights into the DNA damage response chromatin remodellers HELLS and CHD6. These breakthroughs were led by Dr. Wilson Nartey, an exceptional postdoctoral fellow (now research associate) in my lab. Here, I present an overview of our chromatin remodelling research, highlighting the regulation of nucleosome remodelling by HELLS and CHD6, with the latter work done in collaboration with Dr. Aaron Goodarzi's lab.

Susan Lees-Miller

Title: Adventures in DNA double strand break repair

Non-homologous End Joining (NHEJ) is the major pathway for the repair of ionizing radiation-induced DNA double strands breaks (DSBs) such as those induced by radiation and many forms of chemotherapy. Over the past 30 years, our lab and many others have contributed to our understanding of this important pathway, but until recently, no one really knew exactly how NHEJ occurred. Now, advances in cryo-electron microscopy have enabled us to see precisely how the multi-protein NHEJ molecular machine detects and repairs DSBs, paving the way for generation of novel therapeutics to enhance the effectiveness of radiation therapy.