Welcome to the

3rd Canadian Symposium on Telomeres and Genomic Integrity

University of Calgary, Calgary Alberta, May 24-27, 2016

We are excited and pleased to welcome you to the 3rd Canadian Symposium on Telomeres and Genomic Integrity. The goal of this symposium is to provide an opportunity for Canadian Researchers working in the broad field of telomeres and genomic instability to meet, exchange ideas, build collaborations, and expose trainees to world-class research. This symposium is essential for the continued development of a strong Canadian Telomere and Genome Integrity community that will help Canada develop and maintain its international reputation in this area and its application to human health.

Sincerely,

The organizers

Jennifer Cobb, Aaron Goodarzi and Tara Beattie
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Direction to Hotel Alma from Calgary International Airport:

- Travel south on Airport Rd NE
- Turn right onto Airport Rd NE W (turns left and becomes Barlow Trail NE N)
- Turn left onto Airport Trail NE W (signs for Alberta 2/City Centre)
- Take the ramp onto AB-2 S
- Take exit 258 for AB-1 W/Trans Canada Highway
- Merge onto 16 Ave NE/Trans-Canada Hwy/AB-1 W
- Take the exit toward McMahon Stadium/The University of Calgary
- Merge onto University Dr NW N
- Turn left onto 24 Ave NW
- Turn right onto University Gate NW

Hotel Alma will be on the left.
Accommodations and Meeting Registration:

All conference participants will be housed at Hotel Alma (University of Calgary, 169 University Gate NW, Calgary, AB T2N 1N4, (403)-220-3203).

Accommodation check in will begin at 3 pm on Tuesday May 24th and check out is by noon on Friday May 27th.

Meeting Registration will take place at the MacEwan Hall Conference Center, Room A/B, University of Calgary (see maps) beginning at 4 pm on Tuesday May 24, 2016.

1. Go to MacEwan Student Centre

2. On the first floor, Mac Hall A and B are across from Jugo Juice (fast food chain).
**Meals:**
All meals during the symposium will be served at the MacEwan Hall Conference Center.

**Parking:**
Guest and public parking is available at the Arts Parkade, adjacent to Hotel Alma. Parking charges apply.

**Emergency Phone Contacts:**
University of Calgary Conference Centre: (403)-210-9375
Tara Beattie: (403)-585-4842
Jennifer Cobb: (403)-473-8411
Shilpa Salgia: (403)-589-8685
**Presentations:**

Both the oral and poster presentations will take place in the MacEwan Hall Conference center.

All oral presentations, including keynotes, will take place in **Room A**. Please bring a copy of your presentation on a memory stick. We will have laptops available for presentations.

The poster sessions will take place in **Room B**. Poster boards will be supplied. Please keep posters no larger than 1m x 1m'.

There will be a competition for best presentations (both oral and posters, for trainees only). The winners will be announced during the banquet on Thursday night.

**These abstracts should not be cited in bibliographies. All information obtained from talks and posters during this meeting should be treated as personal communication and should be cited as such only with consent of the author.**
AGENDA

Tuesday May 24th, 2016

4:00-5:30pm  Registration Desk Open outside MacEwan Conference Centre
5:30-6:45pm  Dinner at MacEwan Conference Centre
6:45-7:00pm  Welcome –Jennifer Cobb
7:00-8:00pm  Introduction: Tara Beattie
Keynote Speaker: Professor Steven Artandi
Stanford University
“Control of telomerase in stem cells and disease”

Wednesday May 25th, 2016

7:30-8:55am  Breakfast and Registration Desk open outside MacEwan Conference Centre

Session 1:  Genome Integrity 1. Chair: Dan Durocher
9:00-9:20am  El Bachir Affar (Maisonneuve-Rosemont Hospital Research Center, University of Montreal)
“Role of Nuclear Exclusion of the Deubiquitinase USP16 in coordinating DNA damage signalling”
9:20-9:40am  Laura Thompson (McManus Lab, University of Manitoba)
“Multiplexed Nuclear Area and Micronucleus Screening Identifies Human Chromosome Instability Genes”
9:40-10:00am  Amélie Fradet-Turcotte (Université Laval, Cancer Research Center, Québec)
“Interplay between readers of RNF168-ubiquitylated chromatin at DNA double strand breaks”
10:00-10:20am  Gareth Williams (Robson DNA Science Centre, University of Calgary)
“Structural insights into the RAD51 paralog complexes reveal molecular details of disease causing mutations”
10:20-10:50am  Coffee and Tea break

Session 2:  Telomere Biology 1. Chair: Chantal Autexier
10:50-11:10am  Raymund Wellinger (Université de Sherbrooke)
“The new kids on the block are actually pretty old”
11:10-11:30am  Tsz Wai (Josephine) Chu (Autexier Lab, Lady Davis Institute, McGill University)
“Human telomerase ‘Insertion in Fingers Domain’ variants can contribute to telomeropathies phenotypes”
11:30-11:50pm  Xu-Dong Zhu (McMaster University)  
“Function of Cdk-dependent TRF1 phosphorylation in Regulating ALT features”

11:50-12:10pm  Jialin Xu (Wong Lab, University of British Columbia)  
“Investigation of Chromosome X Inactivation and Clinical Phenotypes in Female DKC1 Mutations Carriers”

12:20-1:30pm  Lunch

Session 3:  Targeting Cancer. Chair: Ebba Kurz

1:30-1:50pm  Sachin Katyal (University of Manitoba)  
“Modulating DNA repair pathways in the treatment of malignant brain tumours”

1:50-2:10pm  Armin Gamper (University Alberta)  
“Targeting the DNA Damage Response of Cancer Stem Cells to Improve Cancer Therapy”

2:10-2:30pm  Zahra Shire (Weinfeld Lab, University of Alberta)  
“Nano-encapsulaltion of inhibitors of PNKP by polymeric micelles for selective sensitization of cancer cells to DNA damage”

2:30-2:50pm  Abigail-Rachele Mateo (Derry Lab, University of Toronto)  
“CEP-1 is pro-choice for reproductive health in C. elegans”

2:50-3:10pm  Dindial Ramotar (Maisonneuve-Rosemont Hospital Research Center, University of Montreal)  
“C. elegans OCT-2 is a novel uptake transporter and a target for therapeutic drug discoveries”

3:20-3:50 pm  Coffee and Tea break

Session 4:  Genome Integrity 2. Chair: Susan Lees-Miller

3:50-4:10am  Jean-Yves Masson (Centre de recherche du CHU de Québec)  
“Mechanisms of regulation of the tumor suppressor PALB2”

4:10-4:30pm  Mohammad Ali (Hendzel Lab, University of Alberta)  
“Novel Role of RYBP in DNA Double-Strand Break Signalling”

4:30-4:50pm  Yuliang Wu (University of Saskatchewan)  
“Three Little Pigs and the Big Bad Wolf: Three DNA Helicases and Genomic Instability”

4:50-5:10pm  Aaron Goodarzi (Robson DNA Science Centre, University of Calgary)  
“A major IR-induced Artemis substrate is a DSB with an inter-strand cross-linked terminus”

5:20-7:00pm  POSTER SESSION (please refer to page 48 for list of posters)

7:00- 9:00pm  Reception and Dinner

9:00-11:00pm  Social Hour
Thursday May 26th, 2016
7:30-8:55am   Breakfast outside MacEwan Conference Centre
9:00-10:00am   Introduction: Jennifer Cobb

**Keynote Speaker:** Professor Philippe Pasero, French National Centre for Scientific Research
“SAMHD1 links replication stress to innate immunity”

**Session 5:**  DNA Replication. **Chair: Karolin Klement**
10:00-10:20am  Brandon Q. Ho (Brown Lab, Donnelly Centre, University of Toronto)
“Checkpoint Kinases Regulate Protein Re-localization during Replication Stress in Saccharomyces cerevisiae”

10:20-10:40am  Eric Campos (Hospital for Sick Children, University of Toronto)
“Histones and the Inheritance of Epigenetic Information”

10:40-11:10am  Coffee and Tea break

**Session 6:**  Genome Integrity 3. **Chair: Grant Brown**
11:10-11:30am  Peter Stirling (BC Cancer Research Centre, University of British Columbia)
“The Sgs1 and BLM helicases oppose R-loop induced genome instability”

11:30-11:50am  Simran Kaur (Nepveu Lab, McGill University)
“CUT Domains Stimulate APE1 Activity and Confer Resistance to Treatments”

11:50-12:10pm  Nigel O’Neil (Hieter Lab, Michael Smith Laboratories, University of British Columbia)
“Deep mutational profiling of alkylation-induced multinucleotide mutations reveals the signatures of two modes of translesion synthesis”

12:10-12:30pm  Michael Downey (University of Ottawa)
“Polyphosphorylation of lysine as a novel regulator of cell growth and division”

12:30-1:30pm  Lunch

**Session 7:**  Mix Genome Integrity 4/ Telomere Biology 2. **Chair: Jean-Yves Masson**
1:30-1:50pm  Damien D’Amours (IRIC, University of Montreal)
“Fast switches for a slow enzyme: Rescuing the chromosome condensation machinery from the chromatin trap”

1:50-2:10pm  Hugo Wurtele (Maisonneuve-Rosemont Hospital Research Center, University of Montreal)
“Mutations in Replicative Stress Response Pathways Are Associated with S Phase-specific Defects in Nucleotide Excision Repair”
2:10-2:30pm  Michael Yuen (Lansdorp Lab, BC Cancer Research Centre, University of British Columbia)  
“Mapping genomic rearrangements using Strand-Seq”

2:30-2:50pm  Ramiro E. Verdun (University of Miami)  
“AID induces mismatch repair-dependent telomere loss in the absence of uracil N-glycosylase”

2:50-3:10pm  Mireille Tittel-Elmer (Cobb Lab, Robson DNA Science Centre, University of Calgary)  
“Smc5/6 is a telomeric complex that regulates Sir4 binding and TPE”

3:10-3:30pm  Pascal Chartrand (University of Montreal)  
“Cell cycle-dependent spatial segregation of telomerase from sites of DNA damage”

3:30-4:00pm  Coffee and Tea break

Session 8:  Telomere Biology 3. Chair: Raymund Wellinger

4:00-4:20pm  Sabine Hombach-Klonisch (University of Manitoba)  
“Telomere protective functions of HMGA2 in cancer cells”

4:20-4:40pm  Hans Knecht (McGill University)  
“Two opposite patterns of disruption of 3D Telomere-TRF2 interaction occur in classical Hodgkin lymphoma”

4:40-5:00pm  Sabine Mai (University of Manitoba)  
“DNA-poor space in Hodgkin’s lymphoma nuclei traps telomeric fragments”

5:00-5:20pm  Mélanie Criqui (Harrington Lab, IRIC, University of Montreal)  
“Involvement of the DNA damage response in the unstable differentiation of murine embryonic stem cells with short telomeres”

5:20-5:40pm  Karl Riabowol (Robson DNA Science Centre, University of Calgary)  
“Dogs: man’s best friend for telomere biology”

5:40-7:30pm  Reception and Further Poster Viewing (please refer to page 48 for list of posters)

7:30-10:00pm  CONFERENCE BANQUET

9:30 pm  Social Hour at Last Defence Lounge

Friday May 27th, 2016

Breakfast and Departure
2016 KEYNOTE SPEAKERS

Dr. Steven Artandi, PhD
Professor, Stanford University Medical Center
Stanford, California, USA

Dr. Artandi’s research focuses on understanding how telomere dysfunction influences degenerative diseases and cancer. He investigates the cellular and molecular mechanisms that regulate telomere integrity and the enzyme telomerase to further elucidate their role in stem cell function and carcinogenesis.

Dr. Philippe Pasero, PhD
Institute of Human Genetics – CNRS
Montpellier, France

Dr. Pasero’s research is centered on understanding how genomic instability arises in normal cells. Specifically, how replication stress and errors promote the development of cancer and other genetic disorders associated with increased genomic instability.
ORAL PRESENTATIONS
Keynote Presentation
Control of telomerase in stem cells and disease

Steven E. Artandi

Department of Medicine and Biochemistry, Stanford University School of Medicine

One of the invariant features of human cancer is unlimited proliferation, a hallmark conferred by telomerase in 90% tumors. Somatic mutations in the telomerase reverse transcriptase (TERT) gene promoter are highly recurrent in human cancers. Telomerase is also critically important in human stem cells, as evidenced by mutations in telomerase, which contribute to degenerative diseases. Mutations in telomerase-related genes cause a spectrum of tissue failure phenotypes including dyskeratosis congenita, liver cirrhosis, aplastic anemia and pulmonary fibrosis. In many of these tissues, the identity of resident tissue stem cells remains unknown. Based on the central importance of telomerase in maintaining human tissues, we have developed means for identification of stem cell populations in vivo using telomerase reverse transcriptase (Tert) reporter mice. We found that very high telomerase expression is a hallmark of undifferentiated spermatogonia, the mitotic population where germline stem cells reside. We exploited these high telomerase levels as a basis for purifying undifferentiated spermatogonia using fluorescence-activated cell sorting. Telomerase levels in undifferentiated spermatogonia and embryonic stem cells are comparable and much greater than in somatic progenitor compartments. Within the germline, we uncovered an unanticipated gradient of telomerase activity that also enables isolation of more mature populations. Transplantation studies show that germline stem cell activity is confined to the Tert-High cKit− population. Telomere shortening in telomerase knockout strains causes depletion of undifferentiated spermatogonia and eventual loss of all germ cells after undifferentiated spermatogonia drop below a critical threshold. These data reveal that high telomerase expression is a fundamental characteristic of germline stem cells, thus explaining the broad dependence on telomerase for germline immortality in metazoans. Similar approaches to the identification of somatic tissue stem cells will be discussed.
Role of Nuclear Exclusion of the Deubiquitinase USP16 in coordinating DNA damage signaling

El Bachir Affar, Oumaima Ahmed, Nadine Sen, Jessica Gagnon, Nicholas Iannantuono, Salima Daou, Natalia Zamorano, Nazar Mashtalir, Erlinda Fernandez, Helen Yu, Sylvain Meloche

Department of Medicine, University of Montréal and Centre de Recherche, Hôpital Maisonneuve-Rosemont, Montréal, Québec H1T 2M4, CANADA

Histone H2A ubiquitination (H2Aub) is an epigenetic modification emerging as a central determinant in chromatin remodelling, which is prerequisite for various processes including cell proliferation and DNA repair. This posttranslational modification requires the concerted action of the Polycomb Repressive Complex 1 (PRC1) ubiquitin ligase and deubiquitinases (DUBs), enzymes that remove ubiquitin from the substrate. The levels of H2Aub are highly regulated and any aberration of these pathways promote cancer development. Indeed, recent studies identified USP16 DUB enzyme as a regulator of Hox gene expression through H2A deubiquitination. Interestingly, previous studies indicated that USP16 is predominantly cytoplasmic, but yet exerts nuclear functions. The aim of our research is to understand the mechanisms by which USP16 is translocated into the nucleus and coordinate H2A ubiquitination and to determine how defects in this pathway promote cancer development.

Our studies showed that USP16 is mainly cytoplasmic suggesting that nucleo-cytoplasmic transport can play a crucial role in coordinating the function of this DUB in the nucleus. Indeed, we identified a nuclear localization signal (NLS) that when fused to GFP can promote its translocation into the nucleus. Following Leptomycin B treatment, an inhibitor of nuclear export, we observed a protracted accumulation of USP16 in the nucleus, indicating that this DUB is actively excluded from the nucleus by the CRM1/exportin1 system. We note that when USP16 is translocated into the nucleus, H2Aub levels were drastically reduced. We also defined a mechanism whereby a strong nuclear export signal (NES) is responsible for the cytoplasmic retention of USP16. Our data also revealed that USP16 lacking the NES strongly abolishes the double strand DNA repair pathway through downregulation of the DNA damage signalling and ubiquitin ligase RNF168.

Our data provide insights into the complex nature of USP16 subcellular localization and its role in inhibiting H2A ubiquitination and DNA damage response. This study significantly contributes to the understanding of how the ubiquitin system coordinates DNA repair pathways. As the USP16 gene is translocated in leukemia, our study might help identifying novel inhibitors for the treatment of this disease.
Multiplexed Nuclear Area and Micronucleus Screening Identifies Human Chromosome Instability Genes

Laura L. Thompson(1,2), Allison Baergen(1), Zelda Lichtensztejn(2), and Kirk J. McManus(1,2)

(1) Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MB, Canada
(2) Research Institute in Oncology and Hematology, CancerCare Manitoba, Winnipeg, MB, Canada

Chromosome instability (CIN) is an abnormal phenotype frequently observed in cancer that is characterized by the gain or loss of chromosomes or chromosomal fragments. CIN is associated with aggressive, drug resistant cancers, disease recurrence, and poor prognosis. Despite this, the altered genes that underlie CIN remain largely unknown, highlighting the need for studies that seek to identify and characterize novel CIN genes. In this study we developed and utilized two image-based approaches capable of detecting CIN-associated phenotypes following RNAi-based silencing of candidate genes. First, nuclear areas were quantified following silencing, where changes relative to controls reflect DNA content changes that are characteristic of CIN. The second approach monitored micronucleus (MN) formation where increases are indicative of DNA damage or mitotic defects that cause CIN. Traditional cytogenetic approaches (e.g. mitotic chromosome spread analyses) were employed to validate a subset of putative CIN genes identified using these novel approaches.

The above assays were utilized in a multiplexed, high-content screen of 164 human candidate CIN genes in two unrelated, karyotypically-stable cell lines. Collectively, 148 putative human CIN genes were identified. The 10 most promising putative CIN genes were prioritized for subsequent validation based on the number of assays that identified the gene and the strength of the CIN phenotype. Data collected through Western blotting and assessment of mitotic chromosome spreads for numerical and structural chromosomal changes following gene silencing supports the validation of genes including SKP1, NUF2, and SPC24 as bona fide human CIN genes. These findings indicate that this screen is capable of detecting phenotypic changes associated with CIN, and can be utilized to uncover novel human CIN genes. Identification and characterization of CIN genes will provide critical insights into CIN and oncogenesis, as well as identify potential targets that could be exploited in novel, precision medicine approaches for superior cancer treatment.
Interplay between readers of RNF168-ubiquitylated chromatin at DNA double strand breaks

Amélie Fradet-Turcotte(3), Julianne Kitevski-LeBlanc(1), Marcus D. Wilson(2), Stephanie Panier(2), Alexandre Orthwein(2), Marella D. Canny(2), Lewis E. Kay(1), Daniel Durocher(2)

(1) Kay Lab, University of Toronto, (2) Durocher Lab, Lunenfeld-Tanenbaum Research Institute, Toronto, (3) Fradet-Turcotte Lab, CHU de Québec Research Center – Université Laval, Cancer Research Center, Québec

Upon DNA double-strand breaks (DSBs), a series of phosphorylation and ubiquitylation events are initiated on the chromatin surrounding the breaks. Together with histone marks already present on the chromatin, these post-translational modifications create a code that is recognized by DNA repair proteins. We are interested in understanding how the recognition of histone modifications directs the repair of DNA. At DSBs, ubiquitylation of Histone H2A by the E3-ligase RNF168 promotes both its own recruitment and the recruitment of proteins that coordinate DNA repair, such as 53BP1, RAP80, BRCA1 and RNF169. Here, I will present our recent findings on the molecular determinants that drive the interaction of RNF168, RNF169 and 53BP1 with H2A K13- and K15-ubiquitylated nucleosomes, two products of RNF168. Our structural and biochemical studies reveal that RNF168 and RNF169 interact with H2A K13/K15-ubiquitylated nucleosomes through a bi-partite module that is composed of a ubiquitin-binding motif (MIU2) and a LR motif (LRM). Furthermore, our results suggest that the LRM participates in the interaction by docking into a negatively charged surface of the nucleosome called the acidic patch. Importantly, we show that the cooperation between the MIU2 of RNF168 and the LRM of RNF169 enables the recognition of a variety of ubiquitylated nucleosomes, an observation that is in sharp contrast with 53BP1, which strictly interacts with H2AK15ub nucleosomes. Globally, our studies suggest that the specificities of different ubiquitylated-chromatin binding modules contribute to the differential recruitment of DNA repair proteins to RNF168-ubiquitylated chromatin.
Structural insights into the RAD51 paralog complexes reveal molecular details of disease causing mutations

Gareth Williams, SilDas S, Anand A, Schild D, Tainer JA

University of Calgary

Homologous recombinational repair (HRR) is essential for the maintenance of genome stability, repairing highly deleterious double-stranded DNA breaks. Key to this process is the recombinase RAD51, which forms a nucleoprotein filament and catalyzes the search for homology. Five RAD51 paralogs have been identified in humans that mediate HRR: RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3. Interactions between these proteins, and complexes of these proteins with RAD51, are important for HRR. Furthermore, mutations in the RAD51 paralogs have been linked to cancer predisposition in humans, such as RAD51C (FANCO) mutations that predispose to ovarian and breast cancer (Gly125Val and Leu138Phe), as well as a Fanconi anemia-like disorder (Arg258His). Yet, due to difficulties working with the Rad51 paralogs, we have very little knowledge about their functions and how their mutations result in disease phenotypes.

To address the lack of structural information for the RAD51 paralogs I have studied the RAD51C (FANCO) homolog from the hyperthermophilic eukaryote Alvinella pompejana (Alvi). Here I present recent high-resolution crystal structures of Alvi RAD51C domains. These structures provide a molecular framework for understanding how human point mutations may disrupt RAD51C functions to result in either an increased risk of ovarian and breast cancer, or a Fanconi anemia-like disorder. Recently, I have also developed purification schemes for the human RAD51 paralog complexes, which is paving the way for structural analyses of RAD51 paralog assemblies. Combining small-angle X-ray scattering and crystallography will allow molecular models of RAD51 paralog complexes to be built, which will be subsequently tested by the design of separation-of-function mutations to block key protein-protein and protein-DNA interfaces. Collectively, these results will provide a molecular basis from which to understand the biological functions of the RAD51 paralogs.
The new kids on the block are actually pretty old

Raymund J. Wellinger, B. Lemieux, N. Laterreur, A. Perederina, and A.S. Krasilnikov

Université de Sherbrooke and Penn State University

Using a native system to enrich for active budding yeast telomerase RNP followed by mass spectrometry, we have been able to identify new Tlc1-binding proteins that are constitutive moieties of the telomerase holoenzyme. These proteins turned out to be known components of the RNase P and RNase MRP RNPs; two related, highly conserved and essential RNPs that are involved in the processing of tRNA, rRNA and certain mRNAs. Both of these RNPs also contain a catalytic RNA subunit (Rpr1 for RNase P and Nme1 for RNase MRP). We thus now report that the Pop1, Pop6 and Pop7 proteins, but no other subunit of the RNase P/MRP complexes, bind to yeast telomerase RNA and are essential constituents of the telomerase holoenzyme. The trio of proteins associates in vitro and in vivo to a specific and highly conserved RNA domain present in Tlc1. This area is shared by the RNAs of RNase P/MRP and was called P3 domain on those. Indeed, the P3 domains of all three RNAs are fully interchangeable without any loss of function. The results further show that the association of Pop1/Pop6/Pop7 with the Tlc1-P3 domain is required to maintain the essential components Est1 and Est2 on the Tlc1 RNA in vivo. Consistently, addition of purified Pop1 allows for telomerase activity reconstitution with wild type telomerase RNA in vitro. Taken together, our results show that the yeast telomerase RNA contains a P3-domain that is bound by the cognate proteins from RNAaseP/MRP RNPs and that this stabilizing module is essential for in vivo activity. Thus, the same chaperoning module has allowed the evolution of functionally and, remarkably, structurally distinct RNPs, telomerase and RNases P/MRP, from unrelated progenitor RNAs.
Human telomerase ‘Insertion in Fingers Domain’ variants can contribute to telomeropathies phenotypes

Tsz Wai (Josephine) Chu(1,2), Deanna MacNeil(1,3), Yasmin D'Souza(3), Chantal Autexier(1,2,3)

Lady Davis Institute for Medical Research, Jewish General Hospital, McGill University

(1) Lady Davis Institute for Medical Research, Jewish General Hospital, Montreal, Canada, (2) McGill University, Experimental Medicine, Montreal, Canada, (3) McGill University, Anatomy and Cell Biology, Montreal, Canada

Telomerase is a tumor biomarker expressed in ~85% of human cancers. Conversely, telomerase deficiency leads to premature aging diseases and bone marrow failure. In this study, we performed an in depth biochemical and functional analysis to investigate the role of an uncharacterized unique motif within the human telomerase reverse transcriptase domain (hTERT), termed insertion in fingers domain (IFD). We studied four variants associated with premature aging syndromes (hTERT-P721R, -T726M, -P785L and -R811C) and three others generated based on sequence conservation (hTERT-V763S, -V791Y and -L805A). Our data showed that mutations within the IFD affect telomerase activity and repeat addition processivity (RAP), which is the unique ability of telomerase to repeatedly synthesize long stretches of DNA using its short integral RNA template, without dissociating from its telomeric substrate. The shelterin component TPP1 was previously shown to recruit telomerase to the telomeres and when in a complex with POT1, stimulates enzyme activity and RAP. We demonstrated that hTERT-V791 and hTERT-L805 are important residues for TPP1-dependent telomerase catalytic stimulation and recruitment to telomeres while hTERT-V763 is important for maintenance of short telomeres. We also demonstrated that the diseases-associated variants can contribute to disease phenotypes through different mechanisms, including defective DNA synthesis and RAP, impaired interaction with TPP1 and as such, failure for catalytic stimulation and less efficient recruitment to telomeres. Furthermore, in telomerase-negative cells expressing the different variants, with the exception of hTERT-P785L, all hTERT mutant-expressing cells displayed defective cell growth compared to hTERT-WT expressing cells, accompanied by telomere shortening over time, telomere deprotection, activation of the DNA damage at the telomeres and apoptosis. Our data demonstrates the important involvement of the IFD in the mediation of telomerase-specific functions, telomere maintenance and cell survival, which ultimately can contribute to premature aging diseases phenotypes.
Function of Cdk-dependent TRF1 phosphorylation in Regulating ALT features

Xu-Dong Zhu, Florence R. Wilson, Angus Ho, John R. Walker

Department of Biology, McMaster University, 1280 Main St. West, Hamilton, Ontario

About 10-15% of human cancers maintain their telomere length through a homologous recombination (HR)-based mechanism, referred to as alternative lengthening of telomeres (ALT). TRF1, a subunit of the shelterin/telosome complex that also includes TRF2, TIN2, hRap1, TPP1 and POT1, has been implicated as a positive mediator of ALT. It has been reported that TRF1 is SUMOylated and that SUMOylation promotes APB formation (Potts and Yu, 2007). However, whether other types of post-translational modifications such as phosphorylation may regulate ALT activity has remained largely uncharacterized. Previously we have reported that Cdk1 phosphorylates TRF1 on T371 and that this phosphorylation leads to a stable pool of TRF1 that can be recruited to sites of DNA double-strand breaks independently of telomeric DNA to facilitate homologous recombination-mediated repair. This finding promoted us to investigate if Cdk1-dependent TRF1 phosphorylation on T371 may play a role in the regulation of ALT activity.

Through analysis of indirect immunofluorescence, we find that Cdk-dependent phosphorylation of TRF1 on T371 in ALT cells promotes preferential association of TRF1 with dysfunctional telomeres in S and G2 phases independently of its binding to telomeric DNA. TRF1 phosphorylated on T371, referred to as (pT371)TRF1, is a component of APBs. Loss of T371 phosphorylation impairs APB formation and C-circle production, indicative of its important role in promoting ALT activity. We show that (pT371)TRF1 association with APBs is dependent upon ATM and HR-promoting factors Mre11 and BRCA1. (pT371)TRF1 association with APBs is also sensitive to transcription inhibition as well as RNaseH1 in the presence of camptothecin, an inhibitor of topoisomerase I. These results altogether lead us to propose a model that Cdk activity in S and G2 phases controls TRF1 association with APBs and that this association may be triggered by transcription-associated DNA damage, perhaps arising from processing RNA-DNA hybrids at telomeres.
Investigation of Chromosome X Inactivation and Clinical Phenotypes in Female DKC1 Mutations Carriers

Jialin Xu(1), Payal P. Khincha (2), Sharon A. Savage(2), and Judy M.Y. Wong(1)

(1) Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada (2) Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD

Dyskeratosis congenita (DC) is an inherited bone marrow failure and cancer susceptibility syndrome caused by germline mutations in telomere biology genes. Germline mutations in DKC1, which encodes the protein dyskerin, cause X-linked recessive DC and lead to dysfunction dyskerin with reduced telomerase RNA stability and telomere maintenance capacity. Female DKC1 mutation carriers do not typically develop clinical features of DC due to skewed X-chromosome inactivation. However, we identified several female carriers with DC-associated phenotypes, including abnormal skin pigmentation, nail dystrophy, and liver diseases. This study evaluated female DKC1 mutation carriers with DC-associated phenotypes to elucidate molecular features of their mutations, in comparison with unaffected carriers and mutation-negative female controls.

Female DKC1 mutation carriers had normal leukocyte telomere lengths comparable to those of age- and gender-matched negative individuals. We observed skewed X-inactivation in blood, buccal cells and cultured primary fibroblasts in all female DKC1 mutation carriers, regardless of phenotypes. We also observed wild-type levels of dyskerin expression, telomerase RNA accumulation, and pseudouridylation present in all mutation carriers at levels comparable to healthy wild-type controls.

Our study suggests that mechanisms in addition to X chromosome inactivation, such as mosaicism, telomere inheritance and environmental interactions may also contribute to DC-like phenotypes present in female DKC1 mutation carriers. Future studies are warranted to understand the molecular mechanisms associated with the phenotypic variability in female DKC1 mutation carriers, and to identify those at risk of disease.
Modulating DNA repair pathways in the treatment of malignant brain tumours

Sachin Katyal

Dept. of Pharmacology and Therapeutics, University of Manitoba; Research Institute in Oncology and Hematology, CancerCare Manitoba, 675 McDermot Ave, Winnipeg, Manitoba, Canada

Current methods to treat glioblastoma multiforme (GBM) are highly invasive, lead to poor quality-of-life and an abysmal patient survival rate thus making GBMs one of the most difficult-to-treat and deadliest form of all primary CNS malignancies. The targeted use of Topoisomerase-1 (Top1) poisons/inhibitors to sensitize or augment tumour cell killing is a long-standing and powerful tool in cancer therapy. A number of trials utilizing Top1 inhibitors to manage GBM have shown promise.

We have uncovered a novel functional requirement for ATM (Mutated in Ataxia Telangiectasia) in resolving oxidative DNA breaks and Topoisomerase 1-DNA covalent complexes (Top1cc), a DNA-protein intermediate that can generate DNA breaks upon collision with the transcriptional machinery or DNA replication forks. These data have identified Top1cc as the first specific endogenous pathogenic neural DNA lesion associated with loss of ATM and heritable pediatric neurodegenerative disease. Furthermore, biochemical and genetic studies identified collaboration of ATM and TDP1 (Tyrosyl-DNA Phosphodiesterase 1) in the resolution of Top1cc during neurodevelopment.

Methods: We hypothesize that co-inhibition of ATM and TDP1 will sensitize brain tumours to Top1-dependent chemotherapy by augmenting Top1cc levels and anti-tumour success. Genetic and biochemical methods to co-inhibit ATM and TDP1 and improve the efficacy of Top1 poisons include cell-based DNA damage repair and viability assays and in vivo brain tumour regression assays using mouse xenograft models.

Results: We have found that brain tumour cell killing via Top1-dependent inhibition is greatly enhanced through an ATM and TDP1 co-inhibition strategy. We are presently validating these results in vivo.

Conclusions: ATM and TDP1 co-inhibition represents an effective two-pronged approach to chemoradiosensitize CNS tumours. As TDP1 antagonizes Top1cc formation and efficacy of Top1 poisons such as the camptothecin (CPT) cohort of drugs, my findings will improve existing Top1-mediated anti-cancer strategies by enhancing tumour cell killing while reducing clinical doses and patient side-effects.
Targeting the DNA Damage Response of Cancer Stem Cells to Improve Cancer Therapy

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Due to their unrestricted proliferation, the genomic integrity of cancer cells is threatened by DNA damage and replication stress. Furthermore, during tumourigenesis, cells often lose DNA repair mechanisms, leading to increased mutagenesis. These DNA repair defects and/or the increased genotoxic stress make cancer cells heavily dependent on the (remaining) intact DNA Damage Response (DDR) pathways. Radiation and most chemotherapies attempt to kill cancer cells by eliciting DNA damage. Combining these genotoxic treatments with drugs that target specific DDR pathways critical for cancer cell survival promises to selectively increase tumour-specific cell death. Such chemotherapies and radiosensitizers can target DNA repair proteins or, as in the case of ATR, signalling proteins that regulate repair and check points.

A recently developed bioavailable ATR inhibitor (AZD6738 provided to us by Astra Zeneca) is entering phase I clinical studies, based on preclinical studies showing that ATR inhibition in combination with chemo- or radiotherapy increases cell killing in vitro and tumour shrinkage in vivo. While these results are promising, tumour growth delay is a poor prognostic factor for therapy. The eradication of so-called cancer stem cells (CSC) is a more important feature to prevent tumour relapse and metastasis. Unfortunately, this subpopulation of tumour cells often displays particular radioresistance and we postulate that important characteristics of a clinically effective radiosensitizer, besides a favourable therapeutic index, should include the radiosensitization of CSC in particular. Our lab is studying the DDR in CSC with particular focus on the efficacy of ATR inhibition in vivo for tumour radiosensitization.
Nano-encapsulation of inhibitors of polynucleotide kinase/ phosphatase (PNKP) by polymeric micelles for selective sensitization of cancer cells to DNA damage

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The aim of this project is to enhance the outcome of radio and chemotherapy with DNA damaging agents in solid tumours through development of tumour targeted delivery systems for inhibitors of important DNA repair enzyme, polynucleotide kinase/phosphatase (PNKP).

Methods: A library of polysubstituted imidopiperidine compounds, were screened for their PNKP inhibitory activity using a novel assay that prevents release of fluorescent 2-aminopurine deoxyribose phosphate from a hairpin oligonucleotide if PNKP phosphatase is inhibited. Identified hit compounds with potent PNKP inhibitory activity were used for encapsulation in polymeric micelles of different structures. The encapsulated drug levels were measured using HPLC and the cumulative in vitro release was investigated by a dialysis method. The non-specific toxicity of the PNKP inhibitors was tested against HCT 116 cells using MTS assay. Free and encapsulated drugs were also tested for their activity in sensitization of HCT 116 cells to irinotecan or radiation.

Results: Our fluorescent-based assay identified three hit compounds namely A12B4C5, A12B4C60 and A12B4C61 as potent inhibitors of PNKP with Kd values of 0.13 and 0.16 µM, respectively. Efficient loading of A12B4C5, A12B4C60 and A12B4C61 was achieved in polymeric micelles, which showed drug: polymer w/w ratios of 1:40. We also measured 57.7, 63.4, 66.2 % drug release from polymeric micellar formulations of A12B4C5, A12B4C60 and A12B4C61 within 24 h. The potent inhibitors show slight toxicity at doses higher than 20 µM. PNKP inhibitors were able to radio/chemosensitize HCT 116 cells.

Conclusions: Our results point to a potential for encapsulated inhibitors of PKNP for sensitization of colon tumours to DNA damaging chemotherapy and radiation.
CEP-1 is pro-choice for reproductive health in *C. elegans*

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The generation of crossovers during meiotic prophase I is critical for maintaining meiotic fidelity. Previous work has hinted at a role for the p53 family in meiosis but how it functions remain undefined. Here, we uncover a cooperative role for the p53-like protein CEP-1 and the meiotic protein HIM-5 in maintaining genome stability in the *C. elegans* germline. Specifically, CEP-1 and HIM-5 promote crossover formation by coordinating the formation of SPO-11-dependent double strand breaks and their accurate repair. Using cep-1 separation-of-function alleles, we show that cep-1 and him-5 also suppress the inappropriate activation of the non-homologous end-joining (NHEJ) pathway. These roles of CEP-1 are dependent on the upstream DNA damage checkpoint but independent of the downstream core apoptosis pathway. These findings reveal an ancestral role for the p53 family in ensuring meiotic fidelity and establishes CEP-1 as a critical determinant of repair pathway choice.
C. elegans OCT-2 is a novel uptake transporter and a target for therapeutic drug discoveries

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Organic Cation Transporters (OCT) orchestrate the uptake of cationic compounds into the cells. In humans, OCTs are localized in vital organs and believed to be linked to the development of chemoresistance. The nematode Caenorhabditis elegans serves as a model to investigate mammalian OCTs by virtue of its highly conserved biological pathways and the identical functioning of many proteins at the molecular level. We show that the OCT-1 and OCT-2 transporters of C. elegans play a pivotal role in the transport of clinically relevant drugs such as doxorubicin and cisplatin used for treating cancers. We establish an in vivo approach to uncover the transport efficiency of OCT-1 and OCT-2 by monitoring chemotherapy-induced apoptosis in animals that are defective in the major DNA repair pathways. Using a combination of C. elegans mutants, we demonstrate that depletion of both transporters abolishes drug-induced apoptosis. Coupled to this finding, deletion of oct-1 led to the upregulation of oct-2, which in turn stimulated the uptake of toxic compounds with deleterious effects on survival. Furthermore, we identified in silico protein-ligand binding validating that known cationic ligands can be docked avidly in contrast to non-cationic ligands, advocating the selective uptake by OCT-1 and OCT-2 in a given physiological state. These strategies serve as a proof of concept whereby cationic transporters can be exploited to accelerate drug discovery. Preselecting molecules in silico and validating them with our model will provide the tools to increase efficacy of chemotherapeutics, avert chemoresistance and enable screening of newly synthesized molecules using a cost-efficient model system.
Mechanisms of Regulation of the Tumor Suppressor PALB2

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One typical mechanism to promote genomic instability, a hallmark of cancer, is to inactivate tumor suppressors, such as PALB2. It has recently been reported that mutations in PALB2 increase the risk of breast cancer by 8-9 fold. PALB2 was identified BRCA2 interacting protein, essential for BRCA2 anchorage to nuclear structures and for its function in double-strand break repair. Inherited mutations in PALB2 are associated with a predisposition for ovarian, breast and pancreatic cancers. The basis of the tumorigenic potential of PALB2 is thought to be related to functions in homologous recombination. Therefore, the regulation of PALB2 during the DNA damage response and the effect of cancer-causing mutation is of high interest. Two mechanisms of regulation of PALB2 will be presented. The first mechanism regulates PALB2 localization to DNA damage sites in S-phase.

To date, predicting the functional consequences of PALB2 mutations has been challenging as they lead to different aggressive phenotypes. Here, we performed a structure-function analysis of PALB2 using PALB2 truncated mutants (R170fs, L531fs, Q775X and W1038X), and uncovered a second PALB2 regulation mechanism by which cancer cells could drive genomic instability.
Novel Role of RYBP in DNA Double-Strand Break Signaling

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RYBP is a multifunctional protein that plays an important role in various physiological processes including epigenetic regulation of gene expression, proliferation, differentiation and apoptosis. First discovered as a putative polycomb group (PcG) protein, RYBP was shown to bind to a wide range of proteins including transcription factors, pro-apoptotic proteins, ubiquitin and/or ubiquitinated proteins (e.g., uH2A). We sought to investigate a possible role of RYBP in genomic stability and/or DNA damage repair. U2OS cells expressing GFP-tagged RYBP were micro-irradiated and RYBP found to be rapidly displaced from the DNA damage sites. Super-resolution microscopy performed by 3D SIM (structured illumination microscopy) also indicates that endogenous RYBP - although co-localizing on chromatin - is excluded from DNA double-stand break sites after gamma irradiation. RYBP displacement is RNF8 and VCP/p97-dependent. Moreover, overexpression of RYBP interferes with the recruitment of some ubiquitin-dependent repair proteins (e.g. BRCA1 complex and RAD51) to the irradiation-induced foci around DNA double-strand breaks. A gene conversion assay shows that high levels of RYBP attenuate homologous recombination repair, thus sensitize U2OS cells to ionizing radiation. Our data indicate that RYBP may unmask binding sites for BRCA1 in DNA damage and its removal from the DNA double-stand break sites might be important for proper DNA damage response.
Three Little Pigs and the Big Bad Wolf: Three DNA Helicases and Genomic Instability

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Helicases are molecular motors that couple the energy of nucleoside triphosphate hydrolysis to the unwinding and remodelling of structured DNA or RNA. They are involved in virtually all aspects of nucleic acid metabolism, including replication, repair, recombination, and transcription. Currently our lab is focusing on three iron-sulphur domain containing helicases: i) FANCJ, also known as BRIP1 or BACH1, whose mutations are linked to breast cancer, ovarian cancer and Fanconi anemia (FA), ii) ChlR1, also known as DDX11, is associated with a unique genetic disorder known as Warsaw Breakage Syndrome (WABS) that is characterized by sister chromatid cohesion defects, and iii) RTEL1, regulator of telomere elongation helicase 1, participates in telomeric metabolism, mutations of which give rise to glioma, Hoyeraal-Hreidarsson syndrome (HH), and familial interstitial pneumonia (FIP). Our biochemical evidence demonstrated that FANCJ is a G4 DNA helicase, ChlR1 is a triplex DNA helicase, and RTEL1 has strong DNA-dependent ATPase activity but inactive on G4 and triplex substrates. Immunofluorescence revealed that ChlR1 and RTEL1, but not FANCJ, localize in the nucleolus. Currently, we are using CRISPR to knockout these helicases in human cell line, and examine their biological function. Collectively, our results suggest these three DNA helicase have distinct roles in defending genomic stability.
A major IR-induced Artemis substrate is a DSB with an inter-strand cross-linked terminus

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The integrity of our DNA is constantly under threat from prevalent environmental mutagens, including ionizing radiation (IR). One of the most dangerous types of DNA damage caused by IR exposure is the DNA double strand break (DSB), whose failed repair can kill a cell or whose erroneous repair can lead to chromosomal translocations, deletions or amplifications, driving genomic instability – the fundamental hallmark of all cancers. The majority of DSBs are repaired quickly and without error due to mechanisms that rapidly detect and repair DNA damage. However, there are additional, complex damages that preclude simple re-ligation of DNA ends. The Artemis nuclease is necessary for the repair of a subset of IR-induced DSBs associated with heterochromatin, and humans lacking Artemis are hugely sensitive to radiation. However, the precise nature of the IR-induced DSB-associated lesion that is processed by Artemis is not known. We hypothesize that the in vivo substrate of Artemis during DNA damage repair represents a complex structure at slowly-repaired, persisting DSB ends. We will present evidence that Artemis functions to cleave ‘pseudo-hairpin’ ended DSBs generated following radiation exposure, formed by the conflagration of a 2'-deoxycytidine interstrand crosslink with a DSB at the same locale.
SAMHD1 links replication stress to innate immunity

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SAMHD1 is a triphosphohydrolase that restricts infection of non-cycling cells by HIV-1 and other viruses by depleting intracellular dNTP pools and/or by degrading viral genomes. Mutations in SAMHD1 cause Aicardi-Goutières syndrome (AGS), a severe autoimmune disease, and have been implicated in chronic lymphocytic leukemia (CLL), but the molecular mechanisms involved are currently unknown. Here, we report a novel function of SAMHD1 at stalled replication forks that is independent of its dNTPase activity. We show that SAMHD1 promotes the degradation of newly-synthesized DNA in a CycA-Cdk2-dependent manner. This function is epistatic to Mre11 and is counteracted by Rad51, a recombinase loaded by BRCA2 to protect stalled replication forks. Importantly, SAMHD1 is required both for activation of the ATR-Chk1 pathway and for the recovery of stalled forks, qualifying it as a key regulator of the replication stress response. Moreover, we show that nascent DNA accumulates in the cytosol of SAMHD1-depleted cells in a RecQ1-dependent manner and activates a type I interferon response. Together, these data suggests the existence of an unexpected link between the replication stress response and cell-intrinsic initiation of autoimmunity.
Checkpoint Kinases Regulate Protein Re-localization during Replication Stress in *Saccharomyces cerevisiae*

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The replication checkpoint is essential for accurate DNA replication and repair, and maintenance of genomic integrity when a cell is challenged with genotoxic stress. A hallmark of the replication checkpoint is the regulated re-localization of a substantial number of cellular proteins. Our lab recently identified 254 proteins in *Saccharomyces cerevisiae* that changed their subcellular location upon drug-induced replication stress using hydroxyurea (HU) and methyl methanesulfonate (MMS). Here, we assess the role of the essential checkpoint kinase Mec1 in regulating this replication stress-induced protein re-localization. We employ a high-throughput confocal microscopy platform to monitor the movements of GFP-tagged proteins during MMS treatment. Our data suggests that Mec1 regulates one-third of the observed re-localizations during replication stress. Interestingly, we have identified both uncharacterized and known proteins whose subcellular location is regulated by Mec1 independent of its essential downstream effector kinase Rad53. Together, checkpoint kinases regulate a subset of protein re-localizations following replication stress and our results have revealed proteins with novel roles in the replication checkpoint.
Histones and the Inheritance of Epigenetic Information

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Human somatic cells express a number of histone H3 variants, each with surprisingly diverse binding partners despite minimal disparity at the sequence level. The H3.1 variant predominates in dividing cells, yet we are limited in our knowledge of the soluble protein complexes that it forms as it is evicted and deposited onto chromatin.

To gain a better understanding of how histone H3.1 is spatiotemporally chaperoned, we first characterized in depth the soluble proteome of the H3.1 histone: human histone H3.1 was affinity purified from different cellular compartments and biochemically fractionated to isolate protein complexes based on their co-elution with histones, or by virtue of intrinsic enzymatic activities towards histones. Emphasis is here placed on interactions between histones and components of the replication fork, and how nucleosomal histones segregate on replicating DNA to transmit epigenetic information.
The Sgs1 and BLM helicases oppose R-loop induced genome instability

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Dividing cells are constantly under threat from both endogenous and exogenous DNA damaging stresses that can lead to mutations in DNA. One recently recognized potential source of DNA damage is R-loops, which are formed when DNA:RNA hybrids form in genomic DNA. Canonically, DNA repair proteins work downstream of R-loop-induced DNA damage to affect repair and suppress genome instability. Recently, the possibility that some DNA repair pathways actively destabilize R-loops, thus preventing DNA damage has emerged. Here we identify the yeast helicase Sgs1 and its human orthologue BLM as suppressors of R-loop stability. DNA damage and chromosome loss in SGS1 mutants are partially transcription dependent and can be suppressed by ectopic expression of RNaseH1. Mutation accumulation in sgs1delta cells reveals frequent copy-number changes originating at highly-transcribed and R-loop enriched genomic regions. BLM knockdown in human cell lines also induces DNA damage and R-loops.

Our data reveal an unexpected role for transcription in genome instability of cells lacking SGS1 and suggest an R-loop based mechanism. Based on recent linkage of the Fanconi Anemia (FA) pathway to R-loop suppression we favour a model in which the known BLM function in the FA pathway of replication fork protection suppresses R-loop induced genome instability.
CUT Domains Stimulate APE1 Activity and Confer Resistance to Treatments

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CUT domains, also called Cut repeats, are present in three copies in the CUX1 protein. We previously demonstrated that CUT domains stimulate the glycosylase and AP/lyase activities of OGG1. In the present study, we performed in vitro DNA repair assays using purified proteins with fluorophore-based or radiolabeled probes containing various types of base alterations. Using probes containing apurinic/apyrimidinic (AP) sites, we show that CUT domains also stimulate the AP endonuclease 1, APE1. Accordingly, CUX1 knockdown decreases APE1 activity, increases the number of abasic sites in genomic DNA, and sensitizes cancer cells to radiation and the mono-alkylating agent, temozolomide. In contrast, CUX1 overexpression confers resistance to radiation and temozolomide. Importantly, a recombinant protein containing only CUT domains 1 and 2 (C1C2) is devoid of transcriptional activity, yet is sufficient for rapid recruitment to DNA damage, acceleration of DNA repair and increased resistance to treatments. Together these results firmly establish CUX1 as a key accessory factor in base excision repair and implicate the DNA repair functions of CUX1 in the response of cancer cells to DNA damaging agents. CUX1 gene copy number is increased in 70% of cancers and elevated CUX1 expression inversely correlate with patient survival. We propose that the role of CUX1 in accelerating DNA repair explains this unfortunate correlation.
Deep mutational profiling of alkylation-induced multinucleotide mutations reveals the signatures of two modes of translesion synthesis

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Alkylating agents are ubiquitous environmental mutagens and alkylating agents are commonly used as anti-cancer chemotherapeutics. To better understand the consequences of alkylating agent exposure, we have analyzed an incredibly deep dataset of >800,000 alkylating agent-induced mutations that were generated in 1,997 wild-type *C. elegans* strains as part of the Million Mutations Project. The large number of mutations generated in a common genetic background allowed us to derive remarkably detailed mutational profiles for the alkylating agents EMS and ENU. Our analysis has resulted two key observations.

First, we found that alkylation damage results in frequent multinucleotide mutations (MNMs) that are consistent with the error-prone replication activity of translesion polymerases. This suggests that TLS bypass of alkylated DNA may contribute to the frequent MNMs observed in the human genome. We also compared our data to exome sequencing data from tumors that were treated with the alkylating agent temozolomide, and found that temozolomide also created frequent MNMs with characteristics similar to the mutational profiles we observed in the alkylating agent mutagenized *C. elegans*.

Our second key observation was facilitated by the large number of MNMs (>20,000) in the dataset and the mutational biases of EMS and ENU. We were able to use MNM profiles to assign mutational strand and direction to the bypass events and infer the underlying TLS bypass mechanisms. We found distinct mutational signatures consistent with the polymerase switching and gap filling models proposed for TLS suggesting that both modes of TLS are used to bypass alkylation-induced DNA damage in vivo.
Polyphosphorylation of lysine as a novel regulator of cell growth and division

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In both prokaryotic and eukaryotic cells, phosphate is stored in long chains called polyphosphates. These chains have been implicated in blood coagulation, infection, and growth control, but the mechanisms behind these functions have remained elusive. Surprisingly, it was recently found that polyphosphate chains can be attached to lysine residues as a covalent post-translational modification (Azevedo et al., Mol Cell, 2015). ‘Polyphosphorylation’ of lysine occurs non-enzymatically but can be negatively regulated by exophosphatases to impact the protein-protein interactions and localizations of its two known targets - Nsr1 and Top1 - and the enzymatic activity of Top1. We exploited functional genomics tools and a dramatic electrophoretic shift imparted by polyphosphorylation to identify 16 novel targets in yeast. Among the recovered proteins are known regulators of genome stability including two members of the conserved NuA4/Tip60 acetyltransferase complex and a conserved network of proteins implicated in ribosome biogenesis. We have also demonstrated that human versions of several targets can be modified by polyphosphorylation. Moreover, target polyphosphorylation can be modulated by growth conditions and treatment with the TOR inhibitor rapamycin. I will present our recent progress with this novel post-translational modification and discuss the implications of our findings for its exploitation in the treatment of human diseases such as cancer.
Fast switches for a slow enzyme: Rescuing the chromosome condensation machinery from the chromatin trap

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The compaction of genomic DNA into distinct chromosomes is a hallmark of cell division. The initiation of chromosome condensation also marks the beginning of mitosis—or prophase—in most eukaryotic cells. However, the mechanistic basis for the early mitotic activation of chromosome condensation is unknown. Here we show that the early appearance of chromosome condensation during mitosis is explained by a uniquely low threshold requirement for the activity of the key mitotic inducer, Cdk1. We show that chromosome morphology responds quantitatively to modulation of Cdk1 activity, and that a novel chromatin-folding intermediate can be established in the absence of all other mitotic events. Importantly, we identify the condensin complex as the essential target of Cdk1 in this process, and show that it acts in concert with AAA-class ATPases to efficiently promote chromosome condensation during mitosis. Misregulation of these events lead to chromosome segregation defects and lethality. Collectively, our results reveal an unexpected interplay between Cdk1 phosphorylation cycles and the activity of AAA-class ATPases in the remodelling of mitotic chromatin into functional chromosomes.
Mutations in Replicative Stress Response Pathways are associated with S Phase-specific Defects in Nucleotide Excision Repair

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Ultraviolet (UV) light causes highly genotoxic DNA lesions that are removed by Nucleotide Excision Repair (NER). Indeed, NER is critical to prevent UV-associated skin cancers. UV-induced DNA lesions block DNA polymerases during replication, leading to replicative stress and genomic instability. Upon UV irradiation, blocked replication forks activate the Ataxia-Telangiectasia and Rad3 Related (ATR) kinase, which phosphorylates hundreds of substrates, leading to cell-cycle arrest, inhibition of DNA replication origin firing, and stabilization of blocked replication forks. Intriguingly, previous data in human indicated that reduced ATR activity caused profound NER defects specifically in S phase cells. Several model cancer cell lines also presented such S phase-specific NER defects. However, the molecular mechanisms explaining these phenomena remained poorly understood.

Here, we used the model yeast *Saccharomyces cerevisiae* to investigate the molecular basis of NER modulation during S phase in eukaryotes. We present a novel flow cytometry-based assay allowing quantification of NER efficiency as a function of cell cycle in yeast. Using this assay, we demonstrate that deletion of the ATR homolog Mec1 causes NER defects in S phase, and that initiation of DNA replication is a pre-requisite for the manifestation of this defect. S phase NER was also found to be perturbed by mutation of genes encoding factors playing key roles in a variety of DNA damage response pathways, i.e., cell cycle checkpoint, chromatin remodelling and homologous recombination. Our results further highlight a strong correlation between S phase-specific NER defects and elevated recruitment of RPA to chromatin in mutants that are sensitive to replicative stress. Consistently, we demonstrate that modulation of RPA levels strongly influence NER activity in S phase cells. Overall, our data are consistent with a model where mutations in a variety of DNA damage response pathways cause inordinate sequestration of RPA at stalled DNA replication forks, thereby reducing the availability of this factor to complete its essential role in NER. Our findings have important implications for our understanding of UV-induced skin cancer development in humans.
Mapping genomic rearrangements using Strand-Seq

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Historically, structural variations (SV) such as translocations have been difficult to study. Traditional methods lack the effective resolution to determine breakpoints or require a priori knowledge of the type and location of rearrangements. Next generation sequencing has improved our ability to map SV, but requires very deep sequencing to identify reads that detect the breakpoints. Here we use Strand-seq, a novel single cell sequencing technique that preferentially sequences inherited parental template strands to map SV. Strand-seq differentiates Watson and Crick strands, and the changes in strand-state can be used to mark regions of chromosome rearrangements. To validate the use of Strand-seq in a global, unbiased interrogation of SV, we tested a cell line that was characterized by cytogenetics and found to harbour a complex 4-way translocation with 1 known fusion gene. Sequencing with just a single MiSeq lane, we were able to identify each of the 4 translocations and resolve the breakpoints of each translocation to within a ~20kb region, orders of magnitude better than cytogenetic approaches. We will discuss the ability to automate the analysis pipeline, allowing for rapid, high-resolution breakpoint identification and the ability to resolve translocation partners for each breakpoint. Taken together, this approach presents a new vista in cancer genomics, with accurate, high throughput calling of genomic rearrangements present in a plurality of cells, or in a smaller sub-population. Ultimately, we believe using Strand-seq for mapping SV has the potential to become a routine, cost-effective strategy for characterizing cancers, increasing the efficiency and accuracy of cancer diagnosis.
AID induces mismatch repair-dependent telomere loss in the absence of uracil N-glycosylase.

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Immunoglobulin class switch recombination (CSR) is an essential mechanism for the diversification of the humoral immune response through efficient generation of antibody isotypes that mediate the elimination of pathogens. CSR is a programmed deletional recombination event between DNA double strand breaks at the Ig heavy chain gene locus. These DNA breaks are initiated by the mutagenic enzyme activation-induced cytidine deaminase (AID), which converts the cytosine (C) base of deoxycytidine into uracil (U). These guanine-uracil (G:U) mismatches are processed by uracil N-glycosidase (UNG)-dependent base excision repair and MSH2-dependent mismatch repair pathways to yield DNA breaks that can recombine via end-joining mechanisms. Although AID is preferentially targeted to the immunoglobulin locus, as a side effect, it also mutates other genes including proto-oncogenes thereby creating a predisposition for B-cell lymphomas. We now identify a mechanism whereby AID-induced DNA damage and repair at the telomeres can act as a sensor to eliminate B cells at risk of genomic instability. We show that telomeres are off-target substrates of AID and that B cell proliferation depends on protective repair by UNG. In contrast, in the absence of UNG activity, deleterious processing by mismatch repair leads to telomere loss and defective cell proliferation. Indeed we show that UNG-deficiency reduces B cell clonal expansion in the germinal center in mice and blocks the proliferation of tumour B cells expressing AID. Our findings indicate that the ability to repair telomeres targeted by AID acts as a fail-safe mechanism with tumour suppression activity and suggest UNG as a therapeutic target.
Smc5/6 is a telomeric complex that regulates Sir4 binding and TPE

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SMC proteins constitute the core members of the Smc5/6, cohesin and condensin complexes. We demonstrate that Smc5/6 is present at telomeres throughout the cell cycle and its association with chromosome ends is dependent on Nse3, a subcomponent of the complex. Cells harbouring a temperature sensitive mutant, nse3-1, are defective in Smc5/6 localization to telomeres and have slightly shorter telomeres. Nse3 interacts physically and genetically with two Rap1-binding factors, Rif2 and Sir4. When nse3-1 is combined with rif2Δ, there is a partial reversion in telomere elongation resulting from the loss of RIF2 that is independent of homologous recombination (HR). Reduction in telomere-associated Smc5/6 leads to defects in telomere clustering, dispersion of the silencing factor, Sir4, and a loss in transcriptional repression for sub-telomeric genes and noncoding telomeric repeat-containing RNA (TERRA). SIR4 recovery at telomeres is reduced in cells lacking Smc5/6 functionality and vice versa.

However, nse3-1/ sir4Δ double mutants show additive defects for telomere shortening and TPE indicating the contribution of Smc5/6 to telomere homeostasis is only in partial overlap with SIR factor silencing. These findings support a role for Smc5/6 in telomere maintenance that go beyond its canonical role(s) in HR-mediated events during replication and telomere elongation.
**Cell cycle-dependent spatial segregation of telomerase from sites of DNA damage**

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Telomerase can generate a novel telomere at a DNA break, which can lead to gross chromosomal rearrangements with potentially lethal consequences for the cell. Specific pathways, involving the helicase Pif1, Mec1/ATR and resection of DSBs have been found to suppress de novo telomere addition in yeast. Cellular and molecular details about these processes are still not clearly resolved.

We used single-molecule imaging to show that the budding yeast telomerase RNA (TLC1 RNA) is spatially segregated to the nucleolus and excluded from sites of DNA repair in a cell cycle-dependent manner. During the cell cycle, TLC1 RNA accumulates in the nucleoplasm in G1/S, but localizes preferentially to the nucleolus in G2/M. This localization depends on Pif1, which promotes telomerase release from telomeres. In the presence of bleomycin-induced DNA damages, TLC1 remains nucleolar in most G2/M cells. However, in rad52Δ cells, TLC1 RNA molecules accumulate in the nucleoplasm of G2/M cells after DNA damage. Co-localization between TLC1 RNA molecules and DSBs was observed in rad52Δ cells, and this co-localization increased in rad52Δ pif1-m2 cells, leading to increased gross chromosomal rearrangements. The accumulation of TLC1 RNA in the nucleoplasm depends on Cdc13, which rapidly localizes at DNA damage sites. Rad52, but not Rad51, suppresses this process by inhibiting Cdc13 accumulation at DSBs. Moreover, TLC1 RNA trafficking is regulated by the SUMO ligase Siz1, which promotes TLC1 RNA nucleoplasmic localization after DNA damage and gross chromosomal rearrangements in the absence of RAD52. Deletion of Pif1 bypasses the requirement for Siz1 in gross chromosomal rearrangements in absence of RAD52. This study reveals novel roles for Pif1, Rad52 and Siz1-dependent sumoylation in the accumulation of telomerase in the nucleolus, away from sites of DNA repair.
Telomere protective functions of HMGA2 in cancer cells

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The non-histone chromatin binding protein High Mobility Group A2 (HMGA2) has three palindromic AT-hook motifs, which facilitate binding to the minor groove of AT-rich DNA and promote the genome protective functions of HMGA2. HMGA2 improves base excision DNA damage repair (BER) and protects cancer (stem) cells from apoptosis upon treatment with alkylating DNA damaging agents. In addition, HMGA2 protects stalled replication forks in human stem and cancer cells.

We showed that HMGA2 localizes to telomeres in interphase nuclei. HMGA2 binds to the shelterin protein telomere repeat binding factor 2 (TRF2) independent of TRF2-DNA binding or the Rap1-binding domain of TRF2. HMGA2 silencing increases phosphorylation of TRF2 [TRF2Thr188], reduces TRF2-binding to telomere DNA with resulting increased telomere dysfunction as determined by presence of telomere dysfunction-induced foci (TIF), increased number of anaphase bridges and micronuclei. The AT hook domains are dispensable for HMGA2 telomere localization, but they are essential for the HMGA2 telomere protective function.

Currently, it is not known how HMGA2 is recruited to telomeres and which molecular domains are responsible for telomere localization. Using immunofluorescence and immunoprecipitation techniques we show here that HMGA2 co-localizes and interacts with Rap1. Silencing by siRNA of the Rap1 binding partner TRF2 did not affect the interaction between HMGA2 and Rap1. We demonstrate that AT-hook mutant HMGA2 constructs unable to bind to DNA still can interact with Rap-1. Our results suggest that Rap1 binds to the C-terminal region of HMGA2 and actively recruits HMGA2 to telomeres to enhance genomic stability in cancer cells.
Two opposite patterns of disruption of 3D Telomere-TRF2 interaction occur in classical Hodgkin lymphoma

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Classical Hodgkin’s lymphoma (cHL) is a germinal center derived B-cell lymphoma diagnosed by the identification of immunohistologically defined bi- or multinucleated Reed-Sternberg cells (RS cells). Specific changes in the 3D telomere organization, nuclear DNA organization and chromosomal aberrations were identified in the RS precursor, the mononuclear Hodgkin cell (H), albeit to a much lesser extent.

In a post germinal center B-cell in vitro model for EBV-associated cHL, permanent LMP1 expression, as observed in EBV-associated cHL, results in multinuclearity and complex chromosomal aberrations through downregulation of the shelterin protein TRF2. Thus, we hypothesized that the 3D telomere-TRF2 interaction was progressively disturbed during transition from H to RS cells. Applying a combined quantitative 3D TRF2-telomere immune FISH technique (3D TRF2/Telo-Q-FISH) on primary H and RS cells of 14 diagnostic lymph node biopsies (4 EBV-associated cHL), we show here, for the first time, that H and RS cells are characterized by progressive disruption of 3D Telomere-TRF2 interaction, and that two opposite patterns of disruption do occur. Disruption pattern A is defined by massive attrition of telomere signals and a considerable increase of TRF2 signals not associated with telomeres. This pattern is restricted to EBV-negative cHL. Disruption pattern B is defined by telomere de-protection due to an impressive loss of TRF2 signals, physically linked to telomeres. This pattern is typical of LMP1 + EBV-associated cHL but is not restricted to it.

Findings demonstrate that two molecularly disparate pathways converge on the level of 3D Telomere-TRF2 interaction in the formation of RS cells and are a proof of principle for the model of EBV-associated cHL.
DNA-poor space in Hodgkin’s lymphoma nuclei traps telomeric fragments

Sabine Mai and Hans Knecht

University of Manitoba and McGill, Jewish General Hospital

Our recent super-resolution studies have documented that circular DNA-poor space is found in tumour cell nuclei and absent from normal cell nuclei. DNA-poor space refers to regions between nuclear DNA strands and represents nuclear regions devoid of staining with DNA-binding dyes as documented by one of the super-resolution methods (3D-Structured Illumination Microscopy, 3D-SIM).

The data summarized here will focus on DNA-poor space in tumour cell nuclei of Hodgkin’s lymphoma (HL), a lymphoid malignancy of B cell origin. The multinucleated Reed-Sternberg cell (RS), the diagnostic tumour cell of HL, and its precursor, the mono-nucleated Hodgkin (H) cell, make up only 1-3% of total the lymph node mass, the majority of cells being reactive lymphocytes. At present, 80-85% of the patients respond to the current standard of care (a combination chemotherapy +/- radiation), while 15-20% of the patients relapse.

We will present the following data:
1) Quantitative measurements of DNA-poor space visible by super-resolution imaging. H and RS cells differ from each other and from normal lymphocytes.
2) Characterization of circular DNA-poor nuclear areas. They do not represent nucleoli: two typical nucleolus-associated proteins, nucleolin and upstream binding factor (UBF), are not present.
3) The presence of telomeric fragments in DNA-poor space of HL.
4) The presence of promyelocytic leukemia nuclear (PML) proteins in DNA-poor spaces of HL.

We will discuss the implication of these findings for cancer biology and for telomere biology in Hodgkin’s lymphoma.
Involvement of the DNA damage response in the unstable differentiation of murine embryonic stem cells with short telomeres

Mélanie Criqui*, Corinne Saint-Denis*, E. Andrea Mejia Alfaro*, Fabio Pucci+, Lea Harrington*.

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Functional telomeres are critical for the long-term proliferation of many cell types, and for the ability of pluripotent stem cells to self-renew and commit to differentiated cell lineages. For example, short telomeres impair differentiation into neural stem cells or long-term reconstituting hematopoietic stem cells1,2. Previous findings in our laboratory revealed an unexpected property of short telomeres that leads to an increase, rather than decrease, in proliferation. In mouse embryonic stem cells (mESC), critically short telomeres inhibit expression of de novo DNA methyltransferases (Dnmt3a and Dnmt3b) and lead to a decrease in genome-wide DNA methylation3. This perturbation results in the inability to suppress pluripotency factors such as Nanog or Oct4 upon stimulation to differentiate, and a propensity to resume proliferation3. Presently, the mechanisms by which short telomeres elicit these alterations are unknown.

Our aims are to understand how short telomeres evoke this change at a molecular level, and to examine the in vivo consequences of unstable cell differentiation. We established lineage-tracing methods in mESC with short telomeres and showed that ESC that resumed expression of Oct4 after differentiation formed stem-cell tumours (teratomas) in vivo. Furthermore, we showed that p53 inhibition re-established wild-type Nanog levels in mESCs with short telomeres4. Thus, it appears that the proliferative response is mediated via pluripotency gene expression, and could arise via a telomere-induced DNA damage signal. As other groups have suggested an anti-differentiation function of p53 in mESC5, we plan to use CRISPR to test the p53-dependence of the dysregulation of proliferation and cell fate in mESCs with short telomeres. These experiments will address whether DNA hypomethylation and short telomeres are functionally linked via a p53-dependent mechanism.

In many species including humans, telomeric DNA is lost as normal somatic cells replicate due to the end replication problem. When cells lose a critical amount of telomeric DNA, integrity of the shelterin complex is lost, with a corresponding decompacting of the telomere complex. This allows a DNA damage signal to be generated that involves activation of the ATM kinase and the p53 tumour suppressor, resulting in changes in gene expression that induce replicative senescence. Senescence is thought to serve as a barrier to cancer progression since it limits cell replicative capacity. However, short telomeres also result in genomic instability leading to a variety of diseases including heart disease, infectious diseases and a higher rate of cancer incidence and mortality suggesting that telomere length contributes to determining lifespan. Different studies of monozygotic and dizygotic twins and elderly populations have yielded conflicting results, leaving the question of whether telomere length per se affects lifespan, controversial. Use of the otherwise powerful mouse genetic model cannot adequately addressed this issue, because the dynamics of rodent telomeres differ significantly from those of humans. We have begun to characterize and develop a model of telomere dynamics that closely resembles human, and that will allow us to do experimental manipulations not possible in humans. We recently observed a strong correlation between telomere length and lifespan in numerous inbred dog breeds that have average lifespans ranging from 5-14 years. Initial analyses have shown that dog fibroblasts lose telomeric sequence ~7-fold faster than human fibroblasts isolated from similar anatomic locations, and that within at least one breed, the ability to activate telomerase is proportional to telomere length.
POSTER PRESENTATIONS
Poster Session
5:30-7:00pm Wednesday May 25th, 2016
5:40-7:30pm Thursday May 26th, 2016

Posters may be put up during the first coffee break at 3:10pm.
Posters should be approximately 1 x 1 metre in size.

1. **Rami Abou Zeinab** - CHD6: A Potential Regulatory Protein in the Oxidative Stress Response
2. **Nancy Adam** - Telomere length, telomere integrity and DNA damage response
3. **Oumaima Ahmed** - Role of Nuclear Exclusion of the Deubiquitinase USP16 in coordinating DNA damage signaling
4. **Nezeka S. Alli** - Analysis and characterization of gene targets in human cancers exhibiting telomerase dysfunction
5. **Jaspreet Bassi** - 3D nuclear telomere architecture to monitor myeloma patients
6. **Nicole Batenburg** - Functional Analysis of the Role of CSB in DNA Double-Strand Break Repair
7. **Hélène Ben Soussan** - Mechanisms regulating dyskerin post-translational modification
8. **Amanda Bentley-DeSousa** - Expect the Unexpected: Exploring the polyphosphorylation of lysine as a novel post-translational modification
9. **Dan Berger** - DNA double-strand break repair in different neural cell lineages
10. **Erin Bonnell** - Investigating the role of Tbf1 in telomere homeostasis
11. **Sophie Briggs** - The role of telomere biology in brain tumour progression
12. **Sibapriya Chaudhuri** - The role of LSPI motif containing protein partners of protein phosphatase 2A in mitosis
13. **Pauline Douglas** - Investigating mitotic defects in Human DNA-PK CRISPR cells
14. **Johans J. Fakhoury** - DNA Cages, Nanotubes and Sequence Controlled Conjugates: Design and Biological Applications
15. **David Gallo** - The roles and regulation of Rad5, Mgs1 and Pso2 at stressed DNA replication forks in S. cerevisiae
16. **Shella Gilbert-Girard** - Study on Human Herpesvirus 6 integration in telomeres
17. **David Guerit** - Development of live cell imaging assay for human telomerase RNA
18. **Manhong Guo** - A Distinct Triplex DNA Unwinding Activity of ChlR1 Helicase
19. **Angus Ho** - Functional Analysis of TRF1 Phosphorylation in Telomere Length Maintenance Institution
20. **Nicholas Jette** - The Use of Olaparib in the Treatment of Pancreatic Cancer
21. Lucile Jeusset - Generating USP22 knockouts to characterize chromosome instability in a colorectal cancer cell line
22. Karolin Klement - Changes in DNA double strand break (DSB) repair during cellular aging
23. Ebba Kurz - The impact of salicylate, an isoform-specific catalytic inhibitor of human DNA topoisomerase II alpha, on chemotherapeutic efficacy in a murine model of breast cancer
24. Maxime Lalonde - Cohesion and telomeric transcription control
25. Larasati Larasati - The Treslin Domain of Sld3 Mediates binding of Both DDK and Rad53 in Saccharomyces cerevisiae
26. Susan Lees-Miller - Definition of the core non-homologous end joining complex
27. Roderick MacDonald - Synthetic Genetic Array Targeting Yku Implicates the Cdc48-Ufd1-Npl4 Complex in Telomere Homeostasis
28. Deanna MacNeil - Telomerase regulation through post-translational modifications of dyskerin
29. Louis Masclef - Role of the deubiquitinase MYSM1 in the genotoxic stress response
30. Veena Matthew - Regulation of splicing and gene expression by protein sequestration under genotoxic stress
32. Shaun Moore - The molecular mechanism of CHD6 in the preservation of genome stability and cancer prevention
33. Sarah Moradi-Fard - Smc5/6 is a telomeric complex that regulates Sir4 binding and TPE
34. Marina Mostafizar - Targeting DNA repair pathways to enhance treatment against medulloblastoma and malignant glioma
35. Dustin D. Pearson - A major IR-induced Artemis substrate is a DSB with an inter-strand cross-linked terminus
36. Cortt Piett - The Evaluation of the Human Polynucleotide Kinase Phosphatase’s Role in Non-Homologous End Joining and DNA Repair
37. Brooke Rachel - Characterization of the PP1-TACC3 Interaction via the “RVxF” motif and its Role in Mitotic Progression
38. Sarvan Kumar Radhakrishnan - Ku80 C-terminal region is required, in a DNA structure dependent manner, to interact and stimulate DNA-PKcs kinase activity
39. Anthony Rossi - A novel off-target effect of nicotinamide in the response to DNA damage in yeast
40. Hicham Saad - Revealing a role for the genome organization and nuclear bodies in regulating DNA repair
41. Jessica B. Sarthi - STAG2 in transcriptional regulation and Breast Cancer
42. Justin Simms - Bortezomib induced state of BRCAness: combining PARP inhibitors and proteasome inhibitors in Multiple Myeloma
43. Antoine Simoneau - DNA Damage Response Pathways Promote Cell Growth in the Absence of Sirtuin Activity

44. Asha Sinha - Harnessing microglia as a novel glioblastoma therapy

45. Kyle Sorenson - Novel role of the Nej1-Lif1 interaction in preventing genomic instability during Non-homologous end-joining

46. Connor Thompson - Telomerase Inhibition Sensitizes Cancer Cells to Topoisomerase Inhibitors Through Cell-Cycle Stalling

47. Venkatasubramanian Vidhyasagar - Distinct roles of human single strand DNA binding protein hSSB1 and hSSB2

48. Elizabeth Walden - Regulation of cell cycle checkpoints and DNA damage response through Ku70 phosphorylation

49. Chen Wang - Effect of small molecule inhibitors of PARP and PNKP on ATM-deficient colorectal cancer

1- CHD6: A Potential Regulatory Protein in the Oxidative Stress Response

Rami Abou Zeinab, Shaun Moore, Shujuan Fang and Aaron A Goodarzi

Robson DNA Science Centre, University of Calgary,

CHD6 belongs to the Chromatin Helicase DNA binding (CHD) family of chromatin remodelling enzymes. An analysis by the Cancer Genome Atlas (TCGA) indicates that CHD6 gene copy number is expanded in many tumours including: colorectal, breast and lung, all of which arise from oxidatively stressed tissues. We hypothesize that CHD6 is a regulatory protein in the oxidative stress response pathway, and our goal is to investigate the mechanism of regulation and with a view towards reducing the ability of cancer cells to survive oxidative stress. We find that CHD6 protein expression increases rapidly in response to oxidative stress. CHD6 expression also increases following proteasome inhibitor (bortezomib) treatment and is, itself, ubiquitylated, indicative of a proteasome-mediated pathway regulating CHD6 function. CHD6 is predicted to operate within the oxidative stress response pathway involving KEAP1, an E3 ligase that interacts and regulates NRF2, a master transcriptional regulator of antioxidant response. In this pathway, KEAP1 binds, ubiquitylates and induces NRF2 degradation in the absence of oxidative stress and is inactivated by oxidation to enable rapid NRF2 protein stabilization and activity. We have found that CHD6 and KEAP1 interact directly and in a manner requiring the chromodomains of CHD6. Perhaps counter-intuitively, over-expression of KEAP1 stabilizes both endogenous and ectopically expressed CHD6 in multiple cell lines. We will present our ongoing work characterizing CHD6 response and its regulation following oxidative stress.
2- Telomere length, telomere integrity and DNA damage response

Nancy Adam, Tara Beattie, Karl Riabowol

Robson DNA Sciences Centre University of Calgary

Chromosome ends consist of repeated sequences, called telomeres, and the loss of these sequences through every cell division results in the onset of replicative senescence. The shelterin protein complex, in particular TRF2, safeguards and protects telomeres from being recognized as a double-stranded break (DSB) by forming t-loops. When TRF2 dissociates from the telomeres, telomeres become unprotected which triggers activation of the ATM-dependent DNA damage response and non-homologous end-joining. Cancer cells are able to bypass the replicative senescence mechanism through lengthening telomeres by expression of the enzyme telomerase. However, telomerase dysfunction and short telomeres are related to several premature aging diseases like idiopathic pulmonary fibrosis (IPF). Our lab investigated the properties of three telomerase mutations that are associated with IPF. Our study showed that expression of these mutants in human cell lines (HEK293 and BJ) resulted in shorter telomeres than the cell strains that express wild-type telomerase. Paradoxically, even though continuous telomere shortening was observed, the life span of all three cell strains was extended. By using the BJ-cell strains that express the hTERT-mutations as a model, we can get a better understanding of the correlation between telomere integrity, DNA damage response and senescence/proliferation. We hypothesize that telomere length is not solely responsible for cellular senescence, but rather the telomere integrity and stability, which is TRF2-mediated.

To test this hypothesis, I will passage fibroblast strains (Hs68 and BJ, BJ-cells+hTERT mutants) to investigate TRF2 levels, telomere length and telomere integrity using super-resolution optical microscopy techniques (STORM and PALM). To induce an acute increase in available telomeric DNA, I will transfec...
3- Role of Nuclear Exclusion of the Deubiquitinase USP16 in Coordinating DNA Damage Signaling

Oumaima Ahmed, El Bachir Affar, Nadine Sen, Jessica Gagnon, Nicholas Iannantuono, Salima Daou, Natalia Zamorano, Nazar Mashtalir, Erlinda Fernandez, Helen Yu, Sylvain Meloche

Department of Medicine, University of Montréal and Centre de Recherche, Hôpital Maisonneuve-Rosemont, Montréal, Québec H1T 2M4, CANADA

Histone H2A ubiquitination (H2Aub) is an epigenetic modification emerging as a central determinant in chromatin remodelling, which is prerequisite for various processes including cell proliferation and DNA repair. This posttranslational modification requires the concerted action of the Polycomb Repressive Complex 1 (PRC1) ubiquitin ligase and deubiquitinases (DUBs), enzymes that remove ubiquitin from the substrate. The levels of H2Aub are highly regulated and any aberration of these pathways promote cancer development. Indeed, recent studies identified USP16 DUB enzyme as a regulator of Hox gene expression through H2A deubiquitination. Interestingly, previous studies indicated that USP16 is predominantly cytoplasmic, but yet exerts nuclear functions. The aim of our research is to understand the mechanisms by which USP16 is translocated into the nucleus and coordinate H2A ubiquitination and to determine how defects in this pathway promote cancer development.

Our studies showed that USP16 is mainly cytoplasmic suggesting that nucleo-cytoplasmic transport can play a crucial role in coordinating the function of this DUB in the nucleus. Indeed, we identified a nuclear localization signal (NLS) that when fused to GFP can promote its translocation into the nucleus. Following Leptomycin B treatment, an inhibitor of nuclear export, we observed a protracted accumulation of USP16 in the nucleus, indicating that this DUB is actively excluded from the nucleus by the CRM1/exportin1 system. We note that when USP16 is translocated into the nucleus, H2Aub levels were drastically reduced. We also defined a mechanism whereby a strong nuclear export signal (NES) is responsible for the cytoplasmic retention of USP16. Our data also revealed that USP16 lacking the NES strongly abolishes the double strand DNA repair pathway through downregulation of the DNA damage signaling and ubiquitin ligase RNF168.

Our data provide insights into the complex nature of USP16 subcellular localization and its role in inhibiting H2A ubiquitination and DNA damage response. This study significantly contributes to the understanding of how the ubiquitin system coordinates DNA repair pathways. As the USP16 gene is translocated in leukemia, our study might help identifying novel inhibitors for the treatment of this disease.
4- Analysis and characterization of gene targets in human cancers exhibiting telomerase dysfunction

Nezeka S. Alli, Kalpana Kalyanasundaram Bhanumathy, ShuangShuang Li, Frederick S.Vizeacoumar, Franco J., Vizeacoumrar

University of Saskatchewan, Department of Oncology, Cancer Cluster

Telomerase is an enzyme that drives the mechanism responsible for capping the ends of chromosomes after replication. Telomeres maintenance is now recognized as one of the key mechanisms required to maintain genome integrity, as defects in this leads to chromosome instability. In many cancers telomerase expression is up-regulated resulting in rapid cell division and eventually resulting in chromosome instability. Based on its role in cancer progression, we are interested in identifying telomerase synthetic dosage lethal partners that can be targeted for drug therapy for cancer treatments. We employed GM00847 cells which are primary skin fibroblast cells and HT180 cells which were derived from connective tissue and genetically engineered these cells to express TERT, a component of the telomerase enzyme. Such cells have an up-regulation of TERT giving a cancer phenotype and will be used as our model for cancers with telomerase dysregulation. To identify synthetic lethal targets in TERT expressing cell, we conducted a pooled shRNA’s screen coupled with a Microarray analysis. The hits from the Microarray analysis are currently being validated using CRISPR shRNA’s to determine the strongest candidate targets that demonstrate synthetic lethality with high TERT expressing cells.

It is becoming increasingly clear that telomere loss plays an important role in chromosome instability commonly associated with cancer. Exploiting telomere dysfunction by looking at novel synthetic dosage lethality interactions will contribute to our understanding of the molecular mechanism underlying chromosomal instability and telomere maintenance. More importantly, a telomeric synthetic dosage lethality network will capture the genetic dependency of the cancer cells and define molecular vulnerabilities associated with these cells leading to a promising translational research.
5- 3D nuclear telomere architecture to monitor myeloma patients

Jaspreet Bassi(1)(2), Ludger Klewes(1), Rami Kotb(3), and Sabine Mai(1)(2)

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Multiple myeloma (MM) is the second most common haematological cancer. MM is always preceded by monoclonal gammopathy of undetermined significance (MGUS). Although risk factors have been established, it is currently impossible to assess an individual’s risk of cancer progression. New approaches are needed for predicting disease progression, for the evaluation of treatment response, and for monitoring of residual disease. In this study, we examined whether changes in 3D nuclear telomere profiles, that are indicative of genomic (in)stability, can predict disease status. Blood and bone marrow (BM) samples were obtained in a blinded manner from patients with MGUS, solitary plasmacytoma, smoldering myeloma (SMM), and MM at diagnosis or at different stages of treatment / follow-up. 3D quantitative fluorescence in situ hybridization (3D Q-FISH) and analysis of telomeric parameters was performed using a semi-automated program, TeloView, and a fully automated program, TeloScan. We measured telomere signal intensity (length of telomeres), number of telomeric aggregates, nuclear volume, and the overall nuclear telomere distribution (a/c ratio). Our preliminary data suggests an increase in the number of short telomeres with progressed disease state and a decrease in this number with effective treatment. In addition, we find that the telomeric profiles allow for the differentiation of MGUS, SMM, and MM. 3D telomere profiling may be a potential future tool for risk stratification.
Cockayne syndrome (CS) is a rare, segmental premature aging disorder in which the majority of cases are caused by mutations in the gene ERCC6 which encodes Cockayne syndrome group B protein (CSB). CSB is a multifunctional protein implicated in chromatin remodelling, transcription-coupled repair and telomere maintenance. Recently, we have reported that CSB plays a novel role in regulating the choice of DNA double-strand break (DSB) repair pathways [Batenburg et al. (2015) EMBO J]. We have shown that loss of CSB impairs the recruitment of homologous recombination (HR) factors BRCA1, RPA and Rad51 to DSBs, while it promotes the recruitment of non-homologous end joining (NHEJ) factors 53BP1-Rif1 in S and G2 cells. This misregulation between HR and NHEJ leads to an increased sensitivity of CSB null cells towards Olaparib, a PARP inhibitor toxic to cells deficient in HR. However, little is known about how CSB is recruited to sites of DNA damage and what it does at the damage site.

Previously, we have shown that CSB is associated with ionizing radiation (IR)-induced damaged chromatin. IR is known to induce various types of DNA damage, including both single- and double-strand breaks. To investigate the recruitment of CSB to sites of DNA DSBs, we have employed the mCherry-LacI-Folkl system in U2OS cells to generate DSBs. We have successfully shown that both endogenous CSB and exogenously-expressed Myc-tagged CSB are found to be accumulated at sites of Folkl-induced DNA DSBs. These findings demonstrate that CSB is indeed associated with DNA DSBs. We will present our preliminary findings on characterization of the regions of CSB involved in its association with DNA DSBs.
Mechanisms regulating dyskerin post-translational modification

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The nuclear protein dyskerin is essential for assembly, stability and activity of H/ACA ribonucleoproteins as well as the telomerase complex. Previous experiments from our laboratory showed that dyskerin is modified through the attachment of a small protein called Small-Ubiquitin-like-Modifier (SUMO). The control of this modification regulates many cellular processes, including transcription and genome stability via the alteration of protein intracellular localization, stability or activity. We previously made the discovery that certain premature aging dyskeratosis congenita-causing mutations occur in a region within dyskerin predicted to be SUMOylated. Mutations in these highly conserved SUMOylation consensus sites impair telomerase RNA accumulation, telomerase activity and telomere maintenance. However, the mechanisms regulating dyskerin SUMOylation are unknown. Our studies are focused on determining if dyskerin SUMOylation is enriched at certain phases of the cell cycle and on the identification of conditions that enhance dyskerin SUMOylation.

As previously shown, our preliminary results confirm dyskerin expression to increase during G2/M. We also observe an increase in dyskerin SUMOylation during S phase, correlating to the optimal activity and recruitment of telomerase to the telomeres. Different cellular stresses were found to induce or stabilize SUMOylation of other proteins, so we are currently treating cells with different DNA-damaging agents. While some agents decrease dyskerin levels, arsenic increases its SUMOylated state. Using Fluorescence in situ hybridization and immunofluorescence we found that the DNA damage generated by arsenic did not localize to telomeres. To examine effects of telomere-specific DNA damage on dyskerin SUMOylation, expression of a modified telomere-binding protein, TRF1 (Killer-RED-TRF1) will be used to induce localized oxidative DNA damage at telomeres in HEK293 cells stably overexpressing His-SUMO3 and FLAG-dyskerin. We will discuss the progress of our studies to characterize dyskerin SUMOylation. Understanding the mechanisms regulating dyskerin SUMOylation will allow a better grasp of the role of dyskerin SUMOylation in H/ACA ribonucleoprotein and telomere biology.
8- Expect the Unexpected: Exploring the polyphosphorylation of lysine as a novel post-translational modification

Amanda Bentley-DeSousa, Yi-Chieh Tseng, Christine Nwosu, and Michael Downey

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Throughout evolution, organisms from yeast to humans have developed elaborately conserved mechanisms to maintain genome integrity in response to both endogenous and exogenous factors. Genome stability is affected by the modification of key cellular proteins that participate in DNA repair and chromatin remodelling. In particular, lysine residues are targeted by a number of modifications including methylation, acetylation, ubiquitylation, and sumoylation.

Polyphosphorylation is a recently discovered non-enzymatic post-translational modification (PTM) that occurs on lysine residues within poly-acidic, serine and lysine rich (“PASK”) motifs. This PTM was identified only on two yeast proteins (Nsr1 and Top1) and requires synthesis of polyphosphate chains by vacuole-bound Vtc4. Polyphosphorylation results in impaired Nsr1-Top1 interaction, concomitant with changes to protein localization and downregulated Top1 topoisomerase activity.

Using yeast as a model organism, I used high-throughput methods to screen for novel polyphosphorylation targets. I have uncovered 16 novel targets that include a conserved network regulating ribosome biogenesis. Other substrates are involved in chromatin remodelling and the regulation of cell division: Chz1, Eaf7, Hpc2, Vps72, and Rts1. Other work in our lab has demonstrated that human Nucleolin and Nop58 proteins (homologs of yeast targets Nsr1 and Nop58) are polyphosphorylated. Future work will focus on determining the molecular functions of polyphosphorylation and its role at the interface of genome stability and cellular growth. We will also assess whether polyphosphorylation competes with other lysine-based PTMs, like ubiquitylation, to regulate protein stability and turnover under context-specific conditions.
DNA double-strand break repair in different neural cell lineages

Dan Berger, Dr. Aaron Goodarzi, Dr. Jennifer Chan

Arnie Charbonneau Cancer Institute, University of Calgary

Background: Cranial radiotherapy (CRT) is an important and effective treatment for brain cancers and high-risk leukemias, but is strongly associated with treatment-related neurocognitive decline that worsens the younger a patient is treated. While previous studies have implicated impaired neurogenesis, decreased brain white matter and neuroinflammation as consequences of CRT, few studies have investigated DSB repair proficiency, pathway choice and cellular responses to CRT in the developing mammalian brain at the pediatric stage. We hypothesize that cells of distinct neural lineages display differential IR sensitivity over time, and that these differences may be due to the varied engagement of DNA repair signalling.

Results: To examine the DSB repair kinetics and proficiency amongst different neural cell lineages, mouse neural stem cells, and astrocytes, oligodendrocytes and neurons terminally differentiated from mouse neural stem cells were exposed to ionizing radiation. DSB repair was then examined over time and quantified by immunofluorescence microscopy for H2AX and 53BP1. Here, we show that we are able to successful differentiate mouse neural stem cells into distinct neural lineages, and that neurons, astrocytes and mouse neural stem cells repair DSBs in a time-dependent fashion, with slightly different DSB repair profiles.

Conclusions: Overall, further investigation is required to elucidate the cellular mechanism underlying any differences in kinetics. These data, however, suggest there are intrinsic differences in repair kinetics and signalling between different neural cell lineages.
10- Investigating the role of Tbf1 in telomere homeostasis

Erin Bonnell, Raymund J. Wellinger

Université de Sherbrooke

By differentiating chromosomal ends from internal breaks, telomeres prevent DNA damage checkpoint activation and provide protection from inappropriate DNA repair activity that could create genomic instability. The reverse transcriptase telomerase is responsible for telomere elongation and is constitutively active in *Saccharomyces cerevisiae*, making it an ideal organism to study telomere homeostasis. When an essential component of telomerase is removed, such as the templating RNA TLC1, cells enter replicative senescence after about 60 population doublings, with a small subset of the cellular population evading senescence via a recombination-dependent process. Previous studies have indicated that the time of onset of senescence can be influenced by many genetic factors, but not all mechanisms are known. TBF1 is an essential gene that has been implicated in telomere homeostasis but its precise roles at telomeres still largely remains to be elucidated. It is known that Tbf1p binds T2AG3 repeats within subtelomeric regions, sequences in the majority of snoRNA gene promoters, as well as promoters of some protein-coding genes. While analyzing certain new tbf1 mutant alleles, we discovered that the protein could have a much more direct role in telomere stability. Introducing a variety of tbf1 mutants into strains that also lack telomerase (tlc1Δ) causes a dramatic change in the rate of senescence. We will discuss the ramifications of these and other results with novel tbf1 alleles.
11- The role of telomere biology in brain tumour progression

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Introduction: Glioblastoma Multiforme (GBM) is a highly aggressive and devastating brain tumour, which has a median survival of only 15 months following diagnosis. The current standardised treatments for this tumour have been largely unsuccessful due to the vast degree of genetic variability between patients with these tumours. Activity of the enzyme telomerase is present in <70% GBM tumours and is thought to be involved early in their development making it a potential target for therapy. In this study we focus on the role of telomerase in GBM and its role in malignant transformation.

Methods: Human BTIC) and mouse cells derived from the sub-ventricular zone (SVZ) were used. Mouse SVZ cells were cultured differentially in EGF/FGF or PDGF following a protocol developed previously by the Cairncross and Weiss labs in which cells treated with PDGF transform to a GBM like phenotype and those in EGF/FGF do not. qPCR and TRAP assays were performed to analyse the levels of TERT mRNA and Telomerase activity respectively.

Results: All human BTIC cell lines tested showed increased levels of TERT mRNA expression compared to BJ fibroblast controls. This suggests increased hTERT levels in the cells which was confirmed via TRAP assay showing increased telomerase activity in all cell lines tested. Transformed and untransformed mouse cell lines showed varying degrees of telomerase activity by TRAP. Interestingly, the 16P transformed cell line showed no telomerase activity, suggesting this particular line may be employing the ALT telomere maintenance mechanism, independent of telomerase.

Conclusion: All human BTIC cell lines with increased TERT mRNA expression showed increased telomerase activity, supporting the role of telomerase in the maintenance of these tumours. However, the transformed mouse cell lines showed varying telomerase activity suggesting the presence of an ALT mechanism in one cell line. The presence of telomerase activity in one untransformed line also suggest the possible self-immortalisation of these cell is in culture, although it has been confirmed previously that these untransformed lines, even in the presence of telomerase, do not form tumours in mice.
The role of LSPI motif containing protein partners of protein phosphatase 2A in mitosis

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Mitosis, like many other cellular processes, is tightly regulated by several protein phosphorylation events. PP2A-B56 is an important mitotic protein phosphatase which contributes to the fidelity of the process of sister chromatid segregation during mitosis by dephosphorylating certain outer kinetochore proteins and thereby stabilizing attachments between sister chromatids and the microtubules of mitotic spindle. PP2A-B56 is recruited to the mitotic spindle by BubR1, a mitotic protein kinase. The minimal amino acid sequence on BubR1 required for binding PP2A-B56 has been identified to be KLpSPIIE or the ‘LSPI’ motif. Repoman, another important mitotic regulator, also binds to PP2A-B56 through the same motif. We hypothesize that LSPI is a general consensus motif for recruitment of PP2A-B56, particularly during mitosis, and that additional proteins utilize this recruitment sequence. We speculate that recruitment of PP2A-B56 is regulated by the phosphorylation status of this motif and this in turn plays an important role in controlling the progression of the cell cycle. Our preliminary results show a putative LSPI motif is present in more than 100 mitotic regulators and several of them interact with PP2A-B56 in a phosphorylation dependent and isoform dependent manner. Further understanding of how interactions between the target proteins and PP2A-B56 affect mitotic progression and/or mitotic exit will provide valuable insight about the mechanisms of specific recruitment and mitotic functions of PP2A-B56.
13- Investigating mitotic defects in Human DNA-PK CRISPR cells

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DNA-PKcs has a well-established role in repair of DNA DSBs via NHEJ. We and others recently found that DNA-PKcs also plays an important role in mitosis. We recently reported that DNA-PKcs is autophosphorylated at multiple sites in mitosis and localizes to centrosomes in prophase and metaphase and the midbody in cytokinesis. We also showed that DNA-PKcs interacts with PLK1 and is phosphorylated by PLK on S3205 in mitosis. To better understand the role of DNA-PKcs in mitosis we have generated HeLa cells with CRISPR deletion of DNA-PKcs and are currently characterizing the mitotic defects in these cells.
DNA nanotechnology has emerged as an exceptionally programmable tool to organise materials. Most current strategies rely on the assembly of complex DNA scaffolds, often containing hundreds of different strands, and using it to position materials into the desired functional structure. Our research group has developed a different approach to build DNA nanostructures. Starting from a minimum number of DNA components, we create structures that are intrinsically reconfigurable and environmentally responsive. We will show the construction of 3D-DNA host structures, such as cages and nanotubes that encapsulate and selectively release drugs and materials, and accomplish anisotropic 3D-organization. These nanostructures are promising for drug and oligonucleotide delivery. We find that they readily enter mammalian cells. Their 3D-morphology protects them from nuclease degradation and they are able to silence gene expression to a significantly greater extent than their component oligonucleotides. We designed a DNA cube that recognizes a cancer-specific gene product, unzips and open into a two-dimensional structure as a result, thus acting as a conditional drug delivery vehicle.

The rich self-assembly behaviour of DNA, RNA and proteins stems from the sequence control of the monomers on the macromolecule chain. We will describe a simple and high yield synthesis of DNA-polymer conjugates, in which the (non-natural) polymeric block is monodisperse and fully sequence-controlled. The sequence order of monomer units in these new materials plays a major role in determining their self-assembly, as well as their ability for gene silencing and drug encapsulation. The combination of sequence controlled polymers and DNA cages opens up a new parameter space, with unexpected assembly modes. Using these structures, we developed a method to transfer DNA patterns onto other materials, thus beginning to address the issue of scalability for DNA nanotechnology.
15- The roles and regulation of Rad5, Mgs1 and Pso2 at stressed DNA replication forks in *S. cerevisiae*

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We recently identified the complement of proteins that change intracellular localization during the *S. cerevisiae* DNA replication stress response. Of these, 28 form sub-nuclear foci, one of which is Rad5. Rad5 is in the post-replication repair (PRR) pathway that signals to promote replication bypass of blockages through ubiquitination of PCNA. In the current study we define the function of Rad5 in nuclear foci and identify proteins that function with Rad5 in the replication stress response. We found that the DNA dependent ATPase Mgs1 and nuclease Pso2 fail to form nuclear foci in the absence of RAD5. Rad5, Mgs1 and Pso2 foci co-localize and all three proteins physically interact, indicating that they function together in the replication stress response. Rad5, Mgs1 and Pso2 foci form predominantly during S phase, suggesting that foci correspond to sites of stressed or damaged replication forks. Using chromatin immunoprecipitation followed by deep sequencing we found that Rad5, Mgs1 and Pso2 are all recruited to DNA replication forks during HU-induced replication stress. Furthermore, recruitment of Rad5, Mgs1 and Pso2 to nuclear foci and stressed replication forks depends on ubiquitination of PCNA. DNA combing analysis revealed that rad5 null cells have increased replication fork speed during replication stress while mgs1 and pso2 null cells have subtler, but additive, increases in replication fork speed. Taken together these results indicate that Rad5, Mgs1 and Pso2 function together in restricting replication fork progression during HU-induced replication stress. We propose that bypass or repair of stressed replication forks by Rad5-Mgs1-Pso2 slows fork progression while preserving genome integrity.
Study on Human Herpesvirus 6 integration in telomeres

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The human herpesvirus 6 (HHV-6) can integrate its genome into the telomeric region of chromosomes. The mechanisms leading to viral integration have yet to be understood, but homologous recombination (HR) events between the HHV-6 genome extremities, containing (TTAGGG)n repeats, and telomeres of human chromosomes is likely. We hypothesize that HHV-6 integration involves DNA breaks, requires DNA fluidity for HR to occur and alterations in the expression of Shelterin proteins protecting the telomeres. Our objective is to demonstrate that these events are observed during HHV-6 infection.

Trigger of a DNA damage response (DDR), as assessed by γ-H2AX and 53BP1 staining, was observed in the virus’ replication zones. Viral proteins and telomeric sequences also co-localized with DDR. The expression of three mRNAs (TRF1, TRF2, TPP1) coding for Shelterin proteins was increased in infected cells compared to uninfected cells. Increased expression of the TRF2 protein in infected cells was confirmed by Western Blot and immunofluorescence. Next, the effects of BRACO-19 (B19), a drug that stabilizes the telomere extremities, were studied. Using a recently developed HHV-6 integration assay, our results indicate that, in HeLa cells, HHV-6 integration frequency is reduced by 45% in presence of B19. However, in U2OS cells, opposite effects were noted.

The viral DNA appears to be perceived as damaged DNA and trigger DNA repair mechanisms. DNA repair between the viral genome and the telomeres could induce HR, leading to viral integration. The increased expression of the Shelterin complex could suggest an alteration of the protein homeostasis at the telomeres. Furthermore, a loss of DNA fluidity through the use of B19 decreased the frequency of integration of HHV-6, which supports the hypothesis by which integration could be initiated by strand invasion at the telomeres. However, additional tests need to be conducted to understand the divergent results obtained in different cell lines.
17- Development of live cell imaging assay for human telomerase RNA

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Our knowledge on telomerase comes from studies performed on cell populations, which reflect the average activity within the population. Thus little is known about the activity of telomerase in single cells, as it acts on a single telomere. For this purpose, we aim to develop an assay to study telomerase in single living human cells by tagging the RNA component of telomerase hTERC with MS2 stem-loops. The MS2-GFP system consists of a fusion between the RNA of interest to tandem repeats of stem-loops that are recognized by the MS2 coat protein fused to GFP, allowing us to image RNA at the single molecule level.

In our model, 1 or 4 MS2 stem-loops have been inserted either at the 5’ end or in the CR4/5 stem-loop of hTERC. TRAP assays show that the addition of 1xMS2 stem-loops to hTERC does not modify telomerase activity in WI38-VA13 telomerase-negative cells but 4xMS2 does. Northern blot hybridization using hTERC or MS2 probes reveal that hTERC-1xMS2 is properly processed, while hTERC-4xMS2 shows faint extra bands. hTERC is known to accumulate into Cajal bodies, which are important for telomerase assembly. Immunofluorescence (IF) against coilin and fluorescence in situ hybridization (RNA-FISH) against hTERC shows that 1xMS2 does not impair hTERC localization to Cajal bodies. At the opposite, 4xMS2 fused to hTERC disrupt the accumulation of this RNA into Cajal bodies. Altogether, these data show that hTERC-1xMS2 is suitable for live cell imaging experiments.

We have been able to image nuclear hTERC/MS2-GFP foci in living cells using a spinning disk confocal microscope. hTERC molecules are present throughout the nucleus and display single particule behavior, while other accumulate into bigger, almost immobile foci, corresponding to Cajal bodies. FRAP experiments reveal a dynamic interaction between hTERC-1xMS2 and Cajal bodies, with recoveries occurring within minutes. This assay should provide novel insights into the dynamics of telomerase assembly and interaction with telomeres.
18- A Distinct Triplex DNA Unwinding Activity of ChlR1 Helicase

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Mutations in the human ChlR1 (DDX11) gene are associated with a unique genetic disorder known as Warsaw Breakage Syndrome (WABS) characterized by cellular defects in genome maintenance. The DNA triplex helix structures that form by Hoogsteen or reverse Hoogsteen hydrogen bonding are examples of alternate DNA structures that can be a source of genomic instability. In this study, we have examined the ability of human ChlR1 helicase to destabilize DNA triplexes. Biochemical studies demonstrated that ChlR1 efficiently melted both intermolecular and intramolecular DNA triplex substrates in an ATP dependent manner. Compared with other substrates such as replication fork and G-quadruplex DNA, triplex DNA was a preferred substrate for ChlR1. Also, compared with FANCJ, a helicase of the same family, ChlR1’s triplex resolving activity is unique. On the other hand, the mutant protein from a WABS patient failed to unwind these triplexes. A previously characterized triplex DNA-specific antibody (Jel 466) bound triplex DNA structures and inhibited ChlR1 unwinding activity. Moreover, cellular assays demonstrated there were increased triplex DNA content and double strand breaks in ChlR1-depleted cells, but not in FANCJ-/- cells, when cells were treated with a triplex stabilizing compound benzoquinonoxinaline (BQQ), suggesting that ChlR1 melting of triple helix structures is distinctive and physiologically important to defend genome integrity. On the basis of our results, we conclude that the abundance of ChlR1 known to exist in vivo is likely to be a strong deterrent to the stability of triplexes that can potentially form in the human genome.
19- Functional Analysis of TRF1 Phosphorylation in Telomere Length Maintenance

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Telomeric repeat-binding factor 1 (TRF1), a subunit of the shelterin complex, is a multifunctional protein implicated in telomere maintenance, cell cycle progression, separation of sister telomeres and DNA double-strand break repair. TRF1 undergoes extensive post-translational modifications, including phosphorylation, which in turn regulates its cellular localization, its stability and its DNA binding activity. To gain insights into TRF1 phosphorylation, we immunoprecipitated TRF1 and subjected it to mass spectrometric analysis. Threonine at position 271 of TRF1 was identified as a candidate phosphorylation site. To investigate the function of TRF1 phosphorylation on T271, we mutated T271 either to alanine or to aspartic acid, the latter being phosphomimic. Subsequently, we depleted endogenous TRF1 in HeLa cells and complemented TRF1-depleted HeLa cells with the vector alone, Myc-tagged wild type TRF1, Myc-tagged TRF1 carrying a non-phosphorylatable mutation of T271A and Myc-tagged TRF1 carrying a phosphomimic mutation of T271D. Analysis of telomere length dynamics revealed a role of T271 in regulating telomerase-dependent telomere elongation. Preliminary data on the role of T271 in regulating TRF1 stability, cellular localization, telomeric DNA binding activity as well as telomeric association of other shelterin subunits will also
The Use of Olaparib in the Treatment of Pancreatic Cancer

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Poly-ADP ribose polymerase (PARP) inhibitors attenuate the activity of PARP proteins in DNA repair and are particularly potent in cells that are defective in repair of DNA double strand breaks (DSBs) by the Homologous Recombination repair pathway. Our lab previously showed that mantle cell lymphoma and gastric cancer cell lines with depletion, loss, or inactivation of the phosphatidylinositol kinase like protein kinase (PIKK), ataxia telangiectasia mutated (ATM) were sensitive to the PARP inhibitor olaparib and that this effect was most apparent in ATM-deficient cells lacking the tumour suppressor protein, p53. Recently, ATM was reported to be mutated in both sporadic and familial forms of pancreatic cancer. I will show that inactivation of ATM by small molecular inhibitor, sensitizes pancreatic cancer cells to Olaparib. This research could inform future clinical trials and/or provide information to test the potential for using ATM and PARP inhibitors as a novel therapy in pancreatic cancer.
Generating USP22 knockouts to characterize chromosome instability in a colorectal cancer cell line

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Chromosome instability (CIN) is an increase in the rate at which chromosomes or large chromosome fragments are gained or lost. CIN is an aberrant phenotype observed in virtually all cancer types, and it is associated with aggressive tumours, multi-drug resistance and consequently poor patient prognosis. It is suspected to drive oncogenesis by increasing the rate at which oncogenes are gained and tumor suppressors are lost. Despite this, the genetic defects and molecular mechanisms underlying CIN remain poorly characterized. Preliminary data from our laboratory suggest that decreased USP22 (Ubiquitin-Specific Peptidase 22) expression induces CIN. Importantly, hypomorphic USP22 expression is observed in numerous cancer types, including colorectal cancer.

To evaluate the role diminished USP22 expression has in CIN and colorectal cancer, we sought to generate USP22-knockout (KO) cells. Karyotypically stable HCT116 cells were transduced with a Cas9 expression vector and twelve Cas9-expressing individual clones were isolated. Cas9 expression levels were evaluated by indirect immunofluorescence (IIF) and Western blot analysis. In addition, morphology, growth rate and karyotypic stability were assessed and compared with the parental line. One stable clone exhibiting high Cas9 expression was selected and transfected with USP22-targeting CRISPR RNAs, from which two USP22-KO clones were isolated. Changes in nuclear area, a hallmark of CIN, were evaluated in the USP22-KO clones and parental line using fluorescence microscopy. Preliminary data indicates that nuclear areas are altered within cells from the two USP22-KO clonal populations relative to controls.

We have successfully generated USP22-KO clones in a colorectal cancer cell line to study the effect reduced USP22 expression has on CIN. Beyond colorectal cancer, insight gained from this novel model may have broad-spectrum implications for numerous additional cancer types exhibiting hypomorphic USP22 expression.
22- Changes in DNA double strand break (DSB) repair during cellular aging

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Cellular Senescence is a permanent cell cycle arrest induced by telomere shortening or prematurely due to severe stress. Exposure of cells to DNA damaging agents leads to the recognition of the DNA ends as double strand breaks (DSBs). Normally, cells have very efficient mechanisms to repair DSBs. However, an increased number of DSBs have been found in replicative senescent cells as well as prematurely aged cells. It is unclear whether these DSBs originated from endogenous or exogenous damage and why these DSBs persist. Since some studies suggested that even one un-repaired break can trigger senescence, the goal of my project is to understand the underlying mechanisms of why and how DNA repair is altered in senescent cells.

To analyze the capability of senescent cells to repair exogenous DNA damage, DSB repair kinetics after ionizing radiation was investigated. Generally, DSBs induced by 1-4 Gray of ionizing radiation, such as γ-irradiation, are repaired within 48 hours. Young and senescent primary human fibroblasts were irradiated and repair kinetics monitored using the universal DSB marker γH2AX (H2AX phosphorylated at S139). Preliminary results suggest that ~10-15% of induced DSB are not repaired after 48 hours in senescent cells, compared to ~1% in young or quiescent cells. These results indicate that despite repair proteins being abundant in senescent cells, the repair pathways might be compromised or certain key players down-regulated or inactivated, which leads to the accumulation of un-repaired DSBs in senescent cells.
23- The impact of salicylate, an isoform-specific catalytic inhibitor of human DNA topoisomerase II alpha, on chemotherapeutic efficacy in a murine model of breast cancer

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DNA topoisomerase II (TOP2) is a ubiquitous enzyme that is essential for cell survival through its role in regulating DNA topology and chromatid separation. It is the intracellular target of several common chemotherapeutics (TOP2 poisons), treatment with which results in the accumulation of cytotoxic enzyme-linked DNA double-stranded breaks. Non-break-inducing TOP2 catalytic inhibitors have also been described. These agents, however, may alter the efficacy of regimens incorporating TOP2 poisons, such as doxorubicin and etoposide. We previously identified salicylate, the primary metabolite of aspirin, as a novel catalytic inhibitor of TOP2 that attenuates the formation of doxorubicin-stabilized DNA double-stranded breaks and decreases cytotoxicity of in cultured cells following treatment with TOP2 poisons. We have established the mechanism of enzyme inhibition and, unexpectedly, we observe that salicylate selectively inhibits the alpha isoform of human TOP2. To determine the impact of salicylate co-administration on chemotherapeutic efficacy, we have now established a murine model of human breast cancer. Strikingly, we observe that salicylate administered in the days prior to and concurrent with etoposide abolishes the anti-tumour effects of etoposide, decreasing survival to no better than that of saline-treated mice. Given that aspirin is among the most commonly used pharmaceuticals worldwide, with 30-40% of North Americans consuming it daily, our work will guide future research in human populations and may inform clinical guidelines on salicylate use in patients receiving chemotherapy.
Cohesion between sister chromatids is established by the Cohesin complex, a protein ring that entraps both sister chromatids. Cohesin is part of the Structure Maintenance Complexes family and is composed of three main proteins: SMC1, SMC3 and MCD1. Sister chromatids cohesion is established in S phase and maintained until anaphase. A defect in cohesion between sister chromatids can lead to important chromosomic aberrations. The general mechanisms of cohesion have been well studied, but little is known about cohesion homeostasis at telomeres. Using microscopy approaches in *Saccharomyces cerevisiae*, we observed that short telomeres display an absence of cohesion in S phase, while normal length telomeres maintain their cohesion. Chromatin immunoprecipitation studies reveal that telomeric cohesion is established at discrete subtelomeric loci. Genetic analysis suggests a link between telomere length, cohesin binding and diminution of cohesion. Indeed, mutants that increase telomere length (like rif1, rif2 and pif1) rescue cohesion at short telomeres, while mutants that induce shortening of telomeres (like tel1, mre11 or yku) reduce Mcd1 binding at short telomeres. Interestingly, tethering Mcd1 or the cohesin loader Scc2-Scc4 at a short telomere represses transcription of telomeric repeat-containing RNA (TERRA) and subtelomeric genes, suggesting a novel role for telomeric cohesion in transcriptional control. These results reveal a new level of complexity in telomeric length control mechanisms and transcription at telomeres. The link between cohesion and telomeric transcription seems to be conserved during evolution, since cohesin also regulates TERRA expression at telomeres in human cells.
The Treslin Domain of Sld3 Mediates binding of Both DDK and Rad53 in Saccharomyces cerevisiae

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Dbf4-dependent kinase (DDK) and Cyclin-dependent kinase (CDK) are both required for the initiation of DNA replication. Although the two kinase complexes were initially characterized as having different targets, it has since been found that there are intersections between their roles in Saccharomyces cerevisiae. Sld3 appears to be a critical activation target in both kinase pathways. Sld3 is required for Cdc45 loading at origins in G1 phase, and CDK phosphorylated Sld3 also promotes the origin-association of Dpb11, a loading factor for GINS. Previous work has indicated that Sld3 origin association is DDK-dependent. We therefore questioned whether DDK and Sld3 physically interact. We showed through co-immunoprecipitation assays that Sld3 binds Cdc7, the kinase subunit of DDK. Next we divided Sld3 into N-terminal (1-423) and C-terminal (417-668) regions, and identified that this N-terminal fragment was sufficient to bind Cdc7. The N-terminal portion of Sld3 includes a region with homology to its human counterpart, the Treslin domain, that has been previously shown to bind Cdc45, and we further determined that this domain is required, but not sufficient, to bind Cdc7.

In addition to being a key player in DNA replication, Sld3 is also a Rad53 checkpoint phosphorylation target in response to DNA replication stress. Rad53 phosphorylation of Sld3 prevents its association with both Cdc45 and Dpb11. We have found that Rad53 interacts with Sld3 through the Rad53 FHA1 domain. Interestingly, we further found that FHA1, like Cdc7, interacts with the Sld3 Treslin domain. We are currently investigating if this Sld3-Cdc7 association is crucial for Sld3 origin association, and whether this interaction may be targeted by Rad53 following checkpoint activation.
26- Definition of the core non-homologous end joining complex

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Repair of DNA double-strand breaks (DSBs) by non-homologous end joining (NHEJ) in human cells is initiated by the binding of the Ku heterodimer to a DSB, followed by recruitment of the core NHEJ factors, including Aprataxin and Polynucleotide kinase/phosphatase-Like Factor (APLF), DNA-dependent protein kinase catalytic subunit (DNA-PKcs), XRCC4-like factor (XLF), XRCC4 (X4)-DNA ligase IV (L4). How these factors act together to tether, process and ligate DSB ends remains enigmatic. Here, small angle X-ray scattering (SAXS) and mutational analyses show that APLF is largely an intrinsically disordered protein that binds Ku, Ku/DNA-PKcs and X4-L4 within an extended NHEJ core complex. X4 and XLF assemble with Ku heterodimers linked to DNA-PKcs via flexible Ku80 C-terminal regions in a complex stabilized through APLF interactions with Ku, Ku/DNA-PKcs, and X4-L4. The defined, flexible six-protein machine informs NHEJ efficiency, observed biochemistry, evolutionary conservation and assembly of the core NHEJ complex.
27- Synthetic Genetic Array Targeting Yku Implicates the Cdc48-Ufd1-Npl4 Complex in Telomere Homeostasis

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The Ku heterodimer plays a central role in telomere maintenance and telomerase homeostasis in yeast. A toroidal protein, it is capable of binding DNA and RNA strands and structures in a sequence-independent fashion. At telomeres, it participates in end capping, localization regulation, and silencing. Associated with telomerase, it assures nuclear localization and complex stability through binding to a known stem-loop.

In cells lacking Ku, deficiencies in the telomerase holoenzyme result in appreciable phenotypes. Considering this, a high-throughput screen was undertaken to investigate synthetic lethal phenotypes in the absence of the Ku heterodimer. Among a list of potential interactors, the Cdc48-Ufd1-Npl4 complex was implicated as an actor in the maintenance or homeostasis of telomeres or telomerase. Cdc48 and its cofactors are involved in many aspects of cellular metabolism, being most heavily implicated in protein degradation. It is termed a “segregase” for its ability to remove components from large complexes; protein-loaded telomeric DNA and the telomerase holoenzyme are conceivably ideal targets.

Evidence suggests that the Cdc48 complex is acting on protein components of telomerase, namely Est1p. Implications of this action, as well as other roles Cdc48 may be playing in the telomere and telomerase environment will be presented.
Telomerase regulation through post-translational modifications of dyskerin

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Regulation of telomerase may be connected to post-translational modifications like SUMOylation, which involves conjugation of small ubiquitin-like modifiers (SUMO) to lysine residues of target proteins. Analyzing the cellular and molecular consequences of mutations at SUMOylation sites in the telomerase component dyskerin will improve understanding of associated premature aging and blood disorders. Previous work in our lab has demonstrated modifications of dyskerin through SUMOylation, and has indicated that mutations which reduce dyskerin SUMOylation coincide with those found in patients with the premature aging syndrome dyskeratosis congenita (DC). Modifying these SUMOylation sites impairs dyskerin stability, accumulation of human telomerase RNA, activity of telomerase, and maintenance of telomeres. Preliminary data suggest that DC-associated SUMOylation-defective dyskerin variants have impaired binding to hTR relative to wildtype dyskerin. This RNA binding deficiency appears to be specific to hTR, offering a possible explanation for the previously observed decreased hTR accumulation in dyskerin-knockdown cells transiently expressing these variants. Single substitutions at SUMOylation sites in the nuclear localization sequence (NLS) of dyskerin do not substantially affect localization when examined via immunofluorescence. It has been previously reported that single point mutations cannot counteract the localization effects of the entire NLS of dyskerin, while modifying lysine-rich clusters in the NLS can result in mislocalization. Localization of dyskerin is also being assessed by means of telomeric chromatin immunoprecipitation experiments to assess the recruitment of disease-associated SUMO variants to the telomere. Moreover, assembly of SUMOylation-defective variants with known interacting components like NOP10, NHP2, and NAF1 is being examined in vitro. We will discuss the progress of our studies to understand how dyskerin SUMOylation regulates telomerase and telomere function. Characterizing dyskerin and its post-translational modification will ultimately provide a greater understanding of how the telomerase ribonucleoprotein is regulated, and how this regulation is implicated in telomeropathies like DC.
Deubiquitinases (DUBs) are a poorly understood family of enzymes that catalyze the reverse reaction of ubiquitination. These proteins control cell cycle, gene expression, DNA damage response (DDR) and other cellular processes, deregulation of which play crucial roles in cancer development. Accordingly, targeting DUBs might prove to be a promising approach for developing novel anti-cancer therapies. MYSM1 is a nuclear DUB containing a SWIRM domain and a SANT domain endowed with DNA-binding and protein-interacting properties. This enzyme has been described as a major histone H2A DUB regulating hematopoietic development and is also a positive regulator of androgen receptor-induced gene activation in prostate cancer. Other recent studies have shown that MYSM1 depletion results in DNA double-strand breaks induction suggesting its involvement in DDR. Indeed, this DUB is phosphorylated by the ATM kinase in response to genotoxic stress. In order to elucidate the function of MYSM1 in DNA repair, we conducted MYSM1 loss of function experiments in mammalian cultured cells followed by treatments with DNA double-strand breaks inducing agents. We then analyzed H2A ubiquitination levels and DNA repair phenotypes. Our results confirm that MYSM1 is involved in DNA damage signalling. In addition, using a tandem affinity immunopurification approach, we identified novel MYSM1-interacting partners, which might play an important role in DNA repair pathways.
30- Regulation of splicing and gene expression by protein sequestration under genotoxic stress

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The cellular response to stress is coupled to an array of dynamic events. In response to genotoxic stress; DNA repair proteins move to sites of damage, the transcription machinery is modified, RNAs are organized into stress granules and P-bodies and proteins can be sequestered in aggregates. These dynamic changes collaborate to repair DNA, and alter the transcriptome and proteome to enable stress recovery. We identified an unexpected relocalization of the splicing factor Hsh155 to both intranuclear (INQ) and cytoplasmic (CytoQ) sites of protein quality control (PQC) upon exposure to the DNA damaging agent, methyl methanesulfonate (MMS). Hsh155 localization to PQC sites is promoted by molecular chaperones Hsp42 and Btn2 and is suppressed by the action of the Hsp104 disaggregase and the INQ component Apj1. Hsh155 protein is stable after MMS treatment and, like other aggregates; new translation is required for Hsh155 relocalization. Functionally, Hsh155 localization to PQC sites is correlated with a precipitous drop in splicing efficiency. To extend previous microarray studies, we conducted whole proteome analysis after MMS treatment and observed specific depletion of ribosomal proteins, whose mRNA products together command the largest need for spliceosomal function in yeast. Additionally Hsh155 relocalization to PQC is inhibited in cells lacking Sfp1, a transcription factor that controls ribosomal gene expression under stress. We propose a model in which sequestration of Hsh155 is required to regulate splicing activity when ribosomal protein gene synthesis is arrested by genotoxic stress. These analyses reveal a novel function of PQC structures and highlight protein sequestration as a potential tool in the dynamic cellular control of the transcriptome and proteome under stress.
31- Feasibility of ATM status as a predictive marker in non-small cell lung cancer

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Platinum based antineoplastic therapies (platins) are a first line treatment for non-small cell lung cancer (NSCLC) that generate DNA breaks and stimulate DNA damage response pathways. A key mediator of this response is ataxia telangiectasia mutated (ATM), an activator of downstream targets involved in DNA repair, cell cycle arrest, and apoptosis. We hypothesize that platin exposure may invoke ATM signalling and that tumours deficient in ATM may be innately sensitive to platin therapies. However, predicative markers must fulfil two criteria. First, hey must predict sensitivity. Second, they must be reliable and cost effective to measure. It is therefore important to have an efficient measure of ATMicity in NSCLC tissue. To test this, ATMicity was measured using three quantification techniques. Tissue samples from the Tom Baker Cancer Centre were first assessed for ATM protein levels using immunohistochemistry. Second, protein levels were assessed using western blotting techniques. In addition, downstream targets of ATM were probed for ATM pathway activation. Finally, qrt-PCR was utilized to measure transcript levels. Protein abundance by western blot or immunohistochemistry appeared to be the best determiner of ATMicity. Transcript levels determined by qrt-PCR did not show correlation with ATM levels. This suggests that immunohistochemistry may be the most cost effective and reliable determiner of ATMicity.
32- The molecular mechanism of CHD6 in the preservation of genome stability and cancer prevention

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CHD6 (Chromodomain, Helicase, DNA binding 6) is a class III CHD/Mi2 family ATP-dependent chromatin remodelling enzyme that, via interactions with the NFE2-related factor 2 (NRF2), is part of a protein complex that responds to oxidative stress – a key feature of IR exposure – and regulates the expression of many detoxifying enzymes following exposure to reactive oxygen species (ROS), thus maintaining cellular redox homeostasis. CHD6 has raised interest since several human ataxias have linkage map regions that encompass the CHD6 gene locus on chromosome 20q11.1-12. Further, catalytically-inactive CHD6 mutant mice exhibit motor coordination defects most consistent with a cerebellar neuron disorder (i.e. ataxia). Since ataxia is commonly seen in human syndromes lacking normal DNA strand break responses, this raises the possibility that CHD6 contributes to some aspect(s) of the DNA break response.

Data obtained in the lab to date demonstrates that CHD6 protein expression increases substantially within one hour after exposure to tert-butyl hydroperoxide (TBH); this increase in expression appears to occur through stabilization of CHD6. Further, CHD6 is recruited to UV-A laser DNA damage tracks as rapidly as one minute after damage induction. Interestingly, this recruitment is entirely dependent upon the enzyme PARP (Poly-ADP Ribose Polymerase), which rapidly ribosylates DNA to enable the recruitment of many proteins following break induction. Retention of CHD6 at laser tracks is abrogated by inactivating mutations within the chromodomains of CDH6, which may indicate how the enzyme is able to bind DNA. Finally, using the alkaline comet assay to assess DNA single strand break number, the most common lesion induced by ROS, revealed that depletion of CHD6 leads to an increase in DNA damage after hydrogen peroxide treatment. We hypothesize that increased CHD6 expression equates with an enhanced response to oxidative stress-induced DNA damage that is advantageous to tumour survival following ROS exposure.
SMC proteins constitute the core members of the Smc5/6, cohesin and condensin complexes. We demonstrate that Smc5/6 is present at telomeres throughout the cell cycle and its association with chromosome ends is dependent on Nse3, a subcomponent of the complex. Cells harboring a temperature sensitive mutant, nse3-1, are defective in Smc5/6 localization to telomeres and have slightly shorter telomeres. Nse3 interacts physically and genetically with two Rap1-binding factors, Rif2 and Sir4. When nse3-1 is combined with rif2Δ, there is a partial reversion in telomere elongation resulting from the loss of RIF2 that is independent of homologous recombination (HR). Reduction in telomere-associated Smc5/6 leads to defects in telomere clustering, dispersion of the silencing factor, Sir4, and a loss in transcriptional repression for sub-telomeric genes and noncoding telomeric repeat-containing RNA (TERRA). SIR4 recovery at telomeres is reduced in cells lacking Smc5/6 functionality and vice versa.

However, nse3-1/ sir4Δ double mutants show additive defects for telomere shortening and TPE indicating the contribution of Smc5/6 to telomere homeostasis is only in partial overlap with SIR factor silencing. These findings support a role for Smc5/6 in telomere maintenance that go beyond its canonical role(s) in HR-mediated events during replication and telomere elongation.
34- Targeting DNA repair pathways to enhance treatment against medulloblastoma and malignant glioma

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Current methods to treat childhood medulloblastoma are highly invasive and lead to poor quality of life while the three-year survival rate of patients afflicted with malignant glioma remains abysmal (<5%). Recurrence of these highly malignant tumours is pervasive as they can adopt several mechanisms to resist anti-cancer therapeutics including activation and up-regulation of DNA repair pathways that act to resolve DNA damage elicited by radiation and chemotherapeutic agents (chemoradiotherapy). DNA repair inhibitors like Poly ADP-Ribose (PAR) Polymerase (PARPi), DNA dependent protein kinase (DNA-PKι) and Ataxia Telangiectasia Mutated (ATMi) have the potential to sensitize tumour cells to DNA damaging chemoradiotherapy as these inhibitors specifically target single yet critical DNA repair enzymes. In combination with anti-tumour agents, these sensitizers can significantly augment anti-cancer therapeutic success and patient-survival. However, differing tumours have variable expression/activity of these enzymes; therefore their identification is required for an effective anti-cancer strategy.

I will identify mechanisms that selectively modulate cellular DNA repair activity in brain tumours through identification of specific DNA repair pathways that promote MB and MG cell resistance to DNA damaging therapeutics. I will be using existing DNA repair inhibitors and comparative gene expression methodology to identify these specific DNA repair enzymes. I also seek to inhibit these DNA repair enzymes to chemosensitize MB and MG cells to DNA damaging therapeutics in an effort to reverse the resistant phenotype. I hope to translate these findings into pre-clinical models whereby my data may lead to identifying next-generation brain cancer treatment with improved patient survival and quality-of-life.
A major IR-induced Artemis substrate is a DSB with an inter-strand cross-linked terminus

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The integrity of our DNA is constantly under threat from prevalent environmental mutagens, including ionizing radiation (IR). One of the most dangerous types of DNA damage caused by IR exposure is the DNA double strand break (DSB), whose failed repair can kill a cell or whose erroneous repair can lead to chromosomal translocations, deletions or amplifications, driving genomic instability – the fundamental hallmark of all cancers. The majority of DSBs are repaired quickly and without error due to mechanisms that rapidly detect and repair DNA damage. However, there are additional, complex damages that preclude simple re-ligation of DNA ends. The Artemis nuclease is necessary for the repair of a subset of IR-induced DSBs associated with heterochromatin, and humans lacking Artemis are hugely sensitive to radiation. However, the precise nature of the IR-induced DSB-associated lesion that is processed by Artemis is not known. We hypothesize that the in vivo substrate of Artemis during DNA damage repair represents a complex structure at slowly-repaired, persisting DSB ends. We will present evidence that Artemis functions to cleave ‘pseudo-hairpin’ ended DSBs generated following radiation exposure, formed by the conflagration of a 2’-deoxycytidine interstrand crosslink with a DSB at the same locale.
36. The Evaluation of the Human Polynucleotide Kinase Phosphatase’s Role in Non-Homologous End Joining and DNA Repair

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Ionizing radiation (IR) therapy is the predominant form of treatment for a wide variety of cancers, as IR induces numerous variations of genomic lesions which can reduce cell proliferation and growth. The most cytotoxic of these DNA lesions is the double strand break (DSB). A DSB occurs when single stranded breaks (SSBs) occur in close proximity to one another on opposite DNA strands and may result in chromosomal breakage. Importantly, IR-induced DNA strand breaks frequently contain non-ligatable 5’-hydroxyl and/or 3’-phosphate groups. Human polynucleotide kinase/phosphatase (PNKP) exhibits 5’ DNA kinase and 3’ DNA phosphatase activities, making it ideal for converting “dirty” DSB ends to compatible ends prior to ligation. The major pathway for the repair of IR induced DSBs in human cells is non-homologous end joining (NHEJ). PNKP interacts with the NHEJ scaffolding protein XRCC4 in a phosphorylation-dependent manner (Koch et al, EMBO J, 2004), suggesting a mechanism by which PNKP is recruited to IR-induced DSBs, however, its precise role in NHEJ remains enigmatic. The current work examines the interaction between PNKP and XRCC4 and the DSB repair kinetics of PNKP-deficient cells. PNKP’s interaction with XRCC4 is influenced by the extent of DNA damage caused, and by the inhibition of key DNA damage repair kinases. Additionally, live cell imaging is currently underway to assess PNKP’s recruitment/retention kinetics in vivo. DSBR kinetics are assessed through immunofluorescence staining for canonical DSB foci markers. DSBs were quantified in a PNKP isogenic background, and have demonstrated that PNKP deficient cells exhibit a late DSB repair defect in a cell cycle dependent manner. Importantly, this phenotype is rescued with the stable re-incorporation of PNKP into knockdown cells.
37- Characterization of the PP1-TACC3 Interaction via the “RVxF” motif and its Role in Mitotic Progression

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Protein Phosphatase 1 (PP1) is a serine/threonine phosphatase which serves to control many different cellular processes and is regulated by binding different regulatory subunits. These regulatory subunits then target the phosphatase towards its substrates. Here we explore the interaction of a regulatory subunit Transforming Acidic Coiled Coil 3 (TACC3), and it interaction with PP1 via the conserved "RVxF" motif. TACC3 is involved in mitotic spindle assembly, stabilization, and plays an important role in proper chromosome segregation. Interactions between these two proteins is studied both in vivo and in vitro as we look at the role of the residues within the KVTF sequence of TACC3 for PP1 binding and the role of the interaction on mitotic spindle assemble and overall mitotic progression.
Ku80 C-terminal region is required, in a DNA structure dependent manner, to interact and stimulate DNA-PKcs kinase activity

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Non-homologous end joining (NHEJ) is a major DNA double strand (DSB) break repair pathway in mammalian cells. The first step in NHEJ is recognition of DSBs by the Ku heterodimer and subsequent recruitment of DNA-dependent protein kinase catalytic subunit (DNA-PKcs), a serine/threonine protein kinase, to form the DNA-PK complex. The Ku heterodimer consists of 70 and 80 kDa subunits and is conserved throughout evolution. It has been suggested that the extreme C-terminal 14 amino acids of Ku80 is required for DNA-PKcs recruitment and activation. However, another study demonstrated that deletion of the Ku80 C-terminal region (CTR) does not abolish DNA-PKcs activation. Thus, there is considerable ambiguity regarding the role of the Ku80 CTR in DNA-PKcs recruitment and activation.

The aim of this study is to understand the role of Ku80 CTR in NHEJ, with focus on its ability to recruit and activate DNA-PKcs kinase activity. For this, I generated Ku80 C-terminal deletions (Ku80 residues 1-718 and 1-569), cloned them into baculovirus vectors and expressed and purified the corresponding Ku heterodimers from insect cells. In vitro autophosphorylation reactions, in presence of calf-thymus DNA, using purified proteins showed that Ku heterodimer with Ku80 residues 1-569 had significant defects in multiple DNA-PKcs autophosphorylation sites. Surprising results were observed when defined DNA structures such as 25 base pair (bp) blunt ended double stranded (ds) DNA was used. Deletion of the entire Ku80 CTR (residues 570-732) lead to abrogation of DNA-PKcs kinase activity and inability to interact with DNA-PKcs protein. We confirmed these findings using surface plasmon resonance technique, which showed that the DNA-PKcs interacted with the Ku80 full length heterodimer bound to the 25 bp ds DNA, but not with Ku80 C-terminal mutants. Our study shows that the Ku80 C-terminal region is required for DNA-PKcs activation in presence of 25 bp ds DNA structures.
A novel off-target effect of nicotinamide in the response to DNA damage in yeast

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Methyl methanesulfonate (MMS) is a DNA damaging agent that acts by alkylating DNA bases. Alkylation stalls DNA replication forks, stops replication and generates DNA double-strand breaks (DSBs). Unrepaired DNA breaks cause prolonged cell cycle arrest and decreased viability. Using S. cerevisiae as a model system, we found that treatment of cells with nicotinamide, a derivative of niacin (Vitamin B3), increased survival of cells plated on high concentrations of MMS. Nicotinamide is an inhibitor of the evolutionarily conserved sirtuin class of protein deacetylases, and its effects are almost always assumed to occur by inhibiting these proteins.

We tested this assumption by generating a strain lacking all five yeast sirtuins and assaying its sensitivity to varying concentrations of DNA damaging agents in the presence and absence of nicotinamide. Unexpectedly, nicotinamide increased survival of this strain of in the presence of MMS, demonstrating that the observed effect is occurring independently of all sirtuin enzymes. Nicotinamide is also used in the NAD+ salvage pathway, and levels of Pnc1, the first catalyzing enzyme in the NAD+ salvage pathway, increased following MMS exposure. Therefore, we generated a strain lacking either Pnc1 or Npt1, the second catalyzing enzyme of the pathway, in addition to all five yeast sirtuins and assayed their sensitivity to MMS in the presence and absence of nicotinamide. Here again, nicotinamide increased survival. We conclude that nicotinamide acts independently of both yeast sirtuins and the NAD+ salvage pathway to regulate the cellular response to MMS-induced DNA damage.
Revealing a role for the genome organization and nuclear bodies in regulating DNA repair

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The organization of the genome and architecture of the nucleus play important roles in regulating several metabolic processes and are emerging as critical parameters in regulating DNA repair and genome instability. To monitor the repair of DNA damage in a native environment, we are introducing a site-specific double strand break (DSB) at defined loci in the mammalian genome coupled to DNA labels that flank the break. To overcome the side effects of the currently used DNA labels, we have developed a new non-intrusive method for labeling DNA loci in mammalian cells. It is based on a short DNA sequence (1 Kb) carrying bacterial nucleation sites that recruit and spread specific proteins coupled to fluorophores, respectively called ANCH and OR. Two label variants; ANCH3-OR3GFP and ANCH4-OR4GFP form stable fluorescent foci on each side of the inducible ISceI break site.

This system allows us to visualize the dynamic movement of the break ends during repair by tracking the foci in live cells with time-lapsed fluorescence microscopy. We are investigating the kinetics at a break in the different stages of repair including 1. free ends, 2. bridging/resection, and 3. ligation in different chromatin environments throughout the genome and in various repair deficient backgrounds. Our preliminary results after monitoring one side of the break in osteosarcoma cells with a stable integration of the system, showed an important increase in its displacement immediately following the break, most probably reflecting the more mobile free broken DNA end preceding the binding of the bridging/processing factors.
Uncontrolled cell proliferation and deleterious mutations in the genome are hallmarks of cancer. Cancer is associated with defects in the cohesin complex. Research has shown that the disorders associated with cohesin defects (including cancer) might result from misregulation of transcription of important target genes and not due to defects in sister chromatid cohesion. Even minute changes in cohesin levels can affect transcription without any overt cohesion defects.

In this study we will investigate the role of STAG2, a subunit of cohesin frequently mutated or lost in cancer including bladder cancer, glioblastoma, colorectal carcinoma. In breast cancer, reduced expression of STAG2 is associated with an invasive form of breast carcinoma and decreased survival after diagnosis. We will investigate the transcriptional role of STAG2 in breast cancer, as loss of STAG2 does not affect DNA content in most cancers but has been suggested to affect transcription. Also, STAG2 contains the LxxLL motif known to be specific to transcriptional coactivators and mediating their interaction with nuclear receptors like the estrogen receptor alpha (ER). We therefore chose to characterize the role of the STAG2 (Scc3) in transcription utilizing both mammalian breast adenocarcinoma cells (MCF7) and budding yeast (S. cerevisiae). In MCF7 cells, we will be carrying out transcriptional analysis at the TFF cluster and ChIP combined with 3C, also known as ChIP-Loop/ ChIA-PET, to delve deeper into the mechanism of how STAG2 regulates transcription at ER target genes. ChIP-Loop will allow us to examine the function of the LxxLL motif in looping characteristic of transcription at ER target genes. In mammalian cells, we show that STAG2 and previously reported RAD21 regulates the mRNA levels of certain genes in the TFF cluster in an ER-dependent manner. Furthermore, we will utilize yeast to characterize the function of the LxxLL motif. Utilizing the 6-AU sensitivity assay we were able to investigate the effect of the mutation/allele on the transcriptional elongation process shedding more light on the transcriptional role of Scc3. We carried out ChIP-qPCR to further analyze the enrichment of STAG2 and RAD21 at these cluster genes.
42- Bortezomib induced state of BRCAness: combining PARP inhibitors and proteasome inhibitors in Multiple Myeloma

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The use of PARP inhibitors is particularly efficacious in cancers that harbor mutations in BRCA1. The lethality of PARPi on BRCA1 deficient cancers is achieved by exploiting the fact that homologous recombination is compromised and unable to repair single stranded DNA lesions. These single stranded DNA lesions become highly toxic double-strand breaks upon replication, and repair is forced to use the more error prone non-homologous end joining. Although this has proved promising in BRCA1 deficient cancers, PARPi show almost no effect on homologous recombination proficient cancers. It has been shown that in the absence of 53BP1, even BRCA1 deficient cells can resume successful repair by homologous recombination, rescuing them from the effect of PARPi.

In the context of Multiple Myeloma cells that are BRCA1 proficient, the inhibition of the 26S proteasome has shown to sensitize MM cells to the use of PARPi. This effect appears to be specific to MM cells, showing no effect in normal hematological cell lineages, or in other malignancies. Investigation into the possible mediators of this effect showed that in MM, unlike U2OS or HeLa cells, the NHEJ promoting protein 53BP1 can localize to the site of DNA damage after treatment of bortezomib. The ability of MM cells to localize and retain 53BP1 at DSBs in the presence of proteasome inhibition likely inhibits homologous recombination. Additionally, as 53BP1 promotes NHEJ by inhibiting the switch to HR, it is likely that it also enables genotoxic repair by the error prone NHEJ.
To ensure the maintenance of genomic integrity, cells rely on a large assortment of interlinked molecular mechanisms that include the regulation of chromatin structure. Histone deacetylases (HDAC) of the sirtuin family are particularly interesting in this regard as they have been shown to play evolutionarily conserved roles in modulating cellular aging and the DNA damage response. This class of enzyme is also attracting growing interest as molecular targets for the development of novel therapies for human diseases, including cancer and fungal infections. Here, we investigated the response to pan-sirtuin inhibition in Saccharomyces cerevisiae as a means to identify and characterize cellular pathways that are influenced by this class of HDAC.

We demonstrate that sirtuin inhibition by nicotinamide (NAM) in Saccharomyces cerevisiae induces dose-dependent proliferation defects associated with strong activation of DNA damage-induced signalling. We further show that these phenomena result in large part from H3K56 and H4K16 hyperacetylation following inhibition of the sirtuins Hst3/Hst4 and Sir2 respectively. To further investigate the genetic and molecular basis of NAM-induced cell proliferation defects, we screened a collection of barcoded homozygous diploid mutants to identify genes whose deletion influence cell fitness in the presence of NAM. Genes involved in telomere maintenance, DNA damage signaling and homologous recombination were identified as required for growth during NAM exposure. Further characterization revealed that sirtuin-mediated H3K56ac and H4K16ac deacetylation is critical for the response to replicative stress instigated by endogenously produced reactive oxygen species. Our results reveal that sirtuin inhibition by NAM cripples the cellular response to endogenous genotoxic stress, and that survival in the presence of exogenous NAM requires specific DNA damage response pathways. Our results outline the critical importance of chromatin structure in the maintenance of genomic integrity in eukaryotic cells.
44- Harnessing microglia as a novel glioblastoma therapy

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Glioblastoma multiforme (GBM) is an aggressive form of brain cancer with poor patient prognosis. Post-surgical treatment typically includes radio- and/or chemotherapy, however damage to healthy brain tissue leads to severe side effects. Microglia, the immune cells of the brain, when associated with GBM have potential to either participate in tumour elimination (oxidative stress, phagocytosis) or promote tumour growth (trophic factors, cytokines, MMPs). Selective modulation of microglial functions can harness and promote their anti-tumour action, thereby initiating a unique endogenous cancer treatment strategy. We have identified PARP-1, a known DNA repair initiating enzyme, as a key modulator of microglial function. We hypothesize that modulation of PARP-1 activity/expression will allow us to harness brain endogenous immune cells to promote tumour cell elimination and thus promote efficacy of chemoradiotherapy.

The proposed mechanism is complex: 1) PARP-1 inhibition reduces cellular DNA repair thereby sensitizing tumour cells to genotoxic drugs, enforcing microglial phagocytosis of the tumour; 2) PARP-1 inhibition in microglia reduces release of known promoters of tumour growth and neurotoxins, while increasing production of trophic factors; 3) Selective PARP-1 activation in microglia promotes microglial tumour cell attack by increasing oxidative stress and release of pro-inflammatory cytokines. The aims are:

1) To establish the effects of PARP-1 inhibition alone, and in combination with targeted microglial PARP-1 activation, on microglial ability to eliminate GBM cells (tumour cells vs healthy glial cells).
2) To identify how microglial PARP-1 modulation (on/off) impacts GBM elimination in vivo (tumour vs brain).

The ability to harness a patient’s endogenous immune system to attack GBM by promoting both microglial anti-tumour properties and utilizing the PARP-1 inhibitor Olaparib, is a unique anti-tumour strategy with immediate transitional potential.
45- Novel role of the Nej1-Lif1 interaction in preventing genomic instability during Non-homologous end-joining

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DNA Double strand breaks (DSBs) are the most deleterious form of DNA damage and can result in genomic instability and cell death if improperly repaired. Non-homologous end-joining (NHEJ) is the major pathway used to repair these lesions in mammalian cells and is highly conserved in budding yeast. In yeast NHEJ, the Mre11-Rad50-Xrs2 (MRX) complex is rapidly recruited to the break site where it holds the loose ends of DNA using the hook domain at the apex of Rad50. This tethering keeps the broken ends in close proximity to promote efficient and accurate repair of the lesion. Lif1-Dnl4 and Nej1 are also recruited to the DSB and are required for the Dnl4 ligase dependent ligation of the DNA ends. Interestingly, the human homologues of Nej1 and Lif1, XLF and XRCC4 respectively, have been shown to form long helical filaments in vitro consisting of alternating XLF and XRCC4 homodimers that can bridge DNA ends suggesting a possible second function for Nej1-Lif1 during yeast NHEJ.

We characterize two Nej1 mutants, Nej1-F335A and V338A that lose interaction with Lif1. We demonstrate that these mutants have redundancy with Rad50 in NHEJ but this is not due to a redundant role with Rad50 in end tethering. Instead these Nej1 mutants play a role in abrogating resection at the DSB and reducing Sgs1 and Exo1 dependent large deletions from aberrant NHEJ repair. The MRX complex through Rad50 is important for tethering DSB ends, while the Nej1-Lif1 interaction is critical in preventing resection (likely through protecting the ends). Our work contributes to understanding the manner by which DNA repair factors promote efficient DSB repair while maintaining the stability of the genome.
Telomerase Inhibition Sensitizes Cancer Cells to Topoisomerase Inhibitors Through Cell-Cycle Stalling

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Telomerase is the ribonucleoprotein reverse transcriptase that catalyzes the synthesis of TTAGGG nucleotide repeats at the ends of linear chromosomes contributing to proper telomeric structure and cap formation. As telomerase is expressed in 85-90% of human cancers but not in most somatic human cells, its inhibition has been an attractive therapeutic target. Telomerase inhibition by GRN163L (Imetelstat) has previously been observed to potentiate genotoxic stress in a cell-cycle (S/G2) specific manner, through an unknown mechanism.

Using the established colony-forming unit assay, we tested the effects of combining GRN163L and cell-cycle-specific DNA-damaging agents in combination with pharmacological ATM inhibition (ATMi) on MCF-7 breast cancer cells, to assess dependence of telomerase’s cyto-protective function on this DNA-damage repair (DDR) transducer. Additive increased cytotoxicity observed when telomerase inhibition was combined with etoposide and ATMi depended upon the order of treatment addition. This indicates that telomerase-inhibition-induced potentiation of etoposide cytotoxicity depends on functional ATM-dependent DDR activities. We then examined the effects these treatments on cell-cycle progression through FACS, observing an increase in cell population with 4N DNA content following GRN163L treatment in multiple telomerase-positive but not telomerase-negative cell lines that was abrogated by ATMi pretreatment.

Investigating possible causes of these cell-cycle distribution changes we observed that telomerase inhibition induces γH2AX DNA-damage foci in a subset of telomerase-positive cells, but not telomerase-negative primary human fibroblasts. Additional FACS and immunocytochemistry experiments show that the 4N DNA content and foci-positive cells co-stain for Cyclin B1, suggesting that GRN163L-treated cells were stalled, but not arrested at G2/M. Subsequent growth assays support a small cumulative growth disadvantage caused by GRN163L treatment. Our results suggest that treatment with GRN163L sensitizes telomerase-positive cells to cell-cycle specific DNA-damaging agents through an ATM-dependent DNA-damage signal that induces cell-cycle stalling, and may represent a separate mechanism by which telomerase inhibition could affect DNA repair homeostasis.
Distinct roles of human single strand DNA binding protein hSSB1 and hSSB2

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hSSB1 and hSSB2 are two newly identified human single-stranded (ss) DNA binding proteins (SSB). Both hSSB1 and hSSB2 have a conserved oligonucleotide/oligosaccharide-binding-fold domain and a divergent carboxy-terminal domain. We have shown that both hSSB1 and hSSB2 exist as a monomer in solution; however, hSSB1 but not hSSB2 exhibits anomalous behaviour because of its disordered C-terminal tail. Deletion of C-terminal tail diminishes the DNA binding ability and stability of hSSB1. Although both hSSB1 and hSSB2 prefers to bind ssDNA than double-stranded (ds) DNA, hSSB2 has higher affinity for ssDNA than hSSB1. Unlike hSSB1, hSSB2 protein binds and multimerizes on ssDNA, whereas the C-terminal tail is responsible for its multimerization. Both hSSB1 and hSSB2 are able to bind single stranded RNA, with hSSB1 having a higher affinity than hSSB2. Since both proteins form separate complex with integrator complex subunit 3 (INTS3) and C9ORF80, we also evaluated the ssDNA binding of INTS3 and C9ORF80. We found that both INTS3 and C9ORF80 exhibited similar ssDNA binding ability, but with 16-fold lower than hSSB1/2. The BindN program and EMSA results revealed that the C-terminus but not the N-terminus of INTS3 is essential for the ssDNA binding. Moreover, we purified INTS3-hSSB2-C9ORF80 heterotrimeric complex and found it exhibited higher affinity towards ssDNA than ssRNA but 3-fold lower than hSSB2. The biochemical evidence suggests that domains in proteins and individual subunits may lead hSSB1 and hSSB2 complex function in DNA and RNA metabolism differentially.
Regulation of cell cycle checkpoints and DNA damage response through Ku70 phosphorylation

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Ku is the initial DNA binding component of the classical Non-Homologous End Joining (NHEJ) double stranded DNA break (DSB) repair pathway. Recently, an additional function for Ku in signalling in the DNA damage response (DDR) pathway has been identified. The phosphorylation of the S155 position within the von Willebrand A (vWA) domain of Ku70 was identified to play a role in signalling in response to DSBs. Ku70 knockout mouse embryonic fibroblasts (Ku70-/- MEFs) expressing a phosphomimetic Ku70 S155D mutant are hypersensitive to irradiation and have a pronounced growth defect. They also display altered gene regulation, constitutive activation of ATM kinase, and cell cycle arrest at the G1/S and G2/M checkpoints. Through co-immunoprecipitation we showed that Aurora B interacts with the Ku70 S155D mutant and phosphorylated Ku70. Aurora B is a kinase that functions in cell cycle progression through its roles in mitosis and has also been suggested to regulate the G1/S checkpoint. Interestingly, the chemical inhibition of Aurora B in vivo produces the same phenotypes as expression of the Ku70 S155D mutant. Therefore, we hypothesize that phosphorylated Ku70 is able to inhibit Aurora B following DNA damage, mediating sustained activation of the DDR and cell cycle arrest. Currently we are working to determine how phosphorylated Ku70 is able to inhibit Aurora B following phosphorylation using in vitro kinase assays. Additionally, we are using fluorescent microscopy for DNA damage markers and flow cytometry cell cycle analysis to determine which of the DNA damage response or cell cycle arrest response occurs first in cell expressing Ku70 S155D. In the future we hope to determine mediators of the Ku70 S155D interaction with Aurora B. This work could lead to the development of therapeutics able to arrest cancer cells through treatment with a Ku70 S155D peptide or manipulation of the endogenous S155 position phosphorylation.
49- Effect of small molecule inhibitors of PARP and PNKP on ATM-deficient colorectal cancer

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Colorectal cancer is the third most common cancer worldwide, with an estimated 24400 new cases diagnosed in Canada in 2014. Standard treatment of colorectal cancer includes surgery, chemotherapy and radiation therapy. Recently, DNA damage response pathways have emerged as potential therapy for many types of cancers (1). Among the many proteins that are involved in DNA damage response and repair pathways, ATM (Ataxia Telangiectasia Mutated) plays an important role in maintaining genome stability (2). Interestingly, recent studies have shown that ATM is either mutated or deleted in nearly 20% of colorectal cancers, and among which over 50% contain p53 mutations (3). Here, we propose that colorectal cancer containing ATM mutation/deletion or whose ATM activity is inhibited by ATM inhibitors will be sensitive to small molecule inhibitors of poly-ADP ribose polymerase (PARP) and polynucleotide kinase/phosphatase (PNKP). In this study, we show that colorectal cancer cell lines without p53 are more sensitive to ionizing radiation or PARP inhibitor treatments when compared to wild type cell lines. Moreover, cells without p53 are highly sensitive to the combined treatment of PARP inhibitor and ATM inhibitor. We are also characterizing colorectal cancer cells with stable knock down of ATM and testing their sensitivity to PARP and PNKP inhibitors. This study will expand our understanding on the sensitivity of colorectal cancer containing ATM and/or p53 mutations to inhibitors of PARP and PNKP, and promote future applications of small molecule inhibitors for the treatment of ATM-deficient colorectal cancer.

Characterization of G-Quadruplex in Alternate-lengthening-of-telomere (ALT) Human Cancer Cell Lines

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G-quadruplex (GQ) is an alternative nucleic acid tertiary structure, formed from guanine-rich sequences, and is implicated to play multiple roles in genome stability and maintenance, epigenetic modifications and transcription regulation. GQs are enriched in genomic regions of clinical importance, including oncogene promoters and, of special interest to us, telomeres.

Telomere has been a target of interest in the battle against cancer as most cancer cells maintain telomeres through reactivation of reverse transcriptase telomerase. However, 10-15% of cancer cells use the alternate-lengthening-of-telomere (ALT), a telomerase independent mechanism of telomere maintenance through other means such as homologous recombination. Although telomerase inhibitor may be used, it could potentially lead to selection for ALT-positive cancer cells. Most human ALT cells have long heterogeneous telomeric tracts with degenerate repeats leading to a lack of proper telomere chromatin formation. This provides the opportunity for the formation of alternative DNA structures such as GQs which may in turn further disrupt telomeric chromatin homeostasis.

We contend that there are differences in the telomeric-GQ profiles between ALT-positive and ALT-negative cancer cell lines and that such differences may lead to a higher susceptibility to GQ-stabilizing ligands in ALT-positive cell lines. Using confocal microscopy to view cells stained with H2AX- and GQ-specific antibodies, we found significantly stronger basal nuclear staining of both antibodies in ALT-positive (KMST1, GM847) than ALT-negative (MCF7) cell lines. We will discuss our findings of ALT-specific patterns of GQ cytotoxicity, and our current model of how their telomeric structure vulnerability could potentially be exploited in the clinics.
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Acknowledgements

We are very grateful to all the sponsors for making this symposium possible:

We would also like to acknowledge the help of Shilpa Salgia, Jumi Lee and Diana Law for on-site organization.
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