Alberta Genomic Instability and Aging Conference

April 30—May 1, 2008

University of Calgary, Foothills Campus

Tom Baker Cancer Centre CC104

Conference Information

Sponsorship

The first Alberta Genomic Instability and Aging Conference was made possible by support from:

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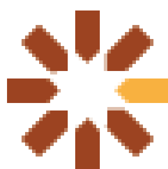
SOUTHERN ALBERTA
Cancer Research Institute

Conference Schedule

Wednesday, April 30, 2008 — Tom Baker Cancer Centre Room CC104

Time	Speaker / Event	Title / Location
9:00 AM	Welcome & Coffee	
9:15 AM	**Dr. William Dunphy**	**Signaling Mechanisms That Protect the Integrity of the Genome**
10:00 AM	Dr. Susan Lees-Miller	Role of phosphorylation in nonhomologous end-joining
10:30 AM	Dr. Shelagh Campbell	In vivo analysis of ATM and ATR-mediated DSB repair and checkpoint functions during female meiosis
11:00 AM	Break	
11:15 AM	Jason Bau (Dr. Ebba Kurz's Lab)	Molecular mechanisms of drug signaling through ATM in human breast cancer cells
11:45 AM	Dr. Frank Jirik	No country for old mice: protein selenocysteine deficiency in chondrocytes results in early lethality
12:15 PM	Lunch	HRIC Atrium
1:30 PM	**Dr. Philippe Pasero**	**Mechanisms preventing replication fork collapse in yeast and human cells**
2:15 PM	Dr. Michael Weinfeld	Processing of DNA strand-break termini
2:45 PM	Dr. Dallan Young	The role of Pnk1 in DNA repair in fission yeast
3:15 PM	Dr. Linda Reha-Krantz	Using a drug-sensitive yeast DNA pol delta to probe check-point arrest and recovery
3:45 PM	Break	
4:00 PM	**Dr. James Keck**	**Structural mechanisms of RecQ DNA helicases**
4:45 PM	Dr. Mike Shultz	Replication stress control of tRNA gene transcription
5:15 PM	Dr. Gordon Chan	The role of the RZZ complex in the kinetochore tension mitotic checkpoint
5:45 PM	Dr. Olga Kovalchuk	Unconfirmed (Title TBA)
6:30 PM	Dinner	HRIC Atrium
7:30 PM	Poster Session	HRIC Atrium

Conference Schedule

Thursday, May 1, 2008 — Tom Baker Cancer Centre Room CC104

Time	Speaker	Title
9:00 AM	**Dr. Joachim Lingner**	**Telomeric repeat containing RNA and telomerase at chromosome ends**
9:45 AM	Nick Ting (Dr. Tara Beattie's Lab)	DNA-PK and Telomere Maintenance
10:00 AM	Haley Wyatt (Dr. Tara Beattie's Lab)	Q169 is an Essential Residue in Human TERT that Modulates MultipleTelomerase Activities
10:15 AM	Dr. Chantal Autexier	Telomere function: Implications for cancer and aging
10:45 AM	Coffee	
11:00 AM	**Dr. Bernard Duncker**	**Preservation of pre-replicative complex integrity in G1 phase**
11:45 AM	Mireille Tittel-Elmer (Dr. Jennifer Cobb's Lab)	The MRX complex maintains the replisome during forks stalling
12:15 PM	Dr. Karl Riabowol	Differential effects of cisplatin on telomere erosion with patient age
1:00 PM	Lunch	HRIC Atrium

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website when it goes live?

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Poster Abstracts

Number	Name	Title/Abstract
1	Laura Baxter	Estrogen induces DNA repair foci in an ATM dependent manner Breast cancer continues to lead in overall cancer incidence among Canadian women with an estimated 22,300 new cases in 2007. Several links between breast cancer incidence and DNA repair have been observed. Numerous reports have shown that blood relatives of A-T patients have a three to seven fold increased risk of breast cancer. Several specific mutations in ATM, including mutations that lead to the A-T phenotype, have been shown to be associated with increased incidence of breast cancer. One possible reason for the increase in risk of breast cancer in particular is the presence of steroid hormones and steroid hormone receptors. It has been reported that metabolism of estrogen induces DNA damage mediated by reactive oxygen species and depurinating DNA adducts. Damage by estrogen has been shown not only to induce base modifications such as 8-hydroxy-2'-deoxyguanosine but has also DNA strand breaks as measured by the alkaline comet assay. These effects have been shown to be both dependent and independent of the estrogen receptor and the exact mechanism of how estrogen induces DNA damage is still unknown. Here we have stably knocked down ATM in the MCF7 breast cancer cell line. We show treatment of MCF7 cells with estrogen results in colocalization of γH2AX, 53BP1 and Ub foci. Formation of these foci is dependent on ATM and its kinase activity as well as the estrogen receptor. Treatment of cells with the ROS scavenger Trolox reduces estrogen induced nuclear foci and treatment of MCF7 cells with the estrogen metabolite 4-OHE2 also induces nuclear foci. These results suggest estrogen and its metabolites induce oxidative DNA damage resulting in the activation of ATM and induction of ATM dependent DNA repair foci.
2	Pinaki Bose	DNA damaged and cell cycle induced localization of ING1 Our lab discovered about twelve years ago a novel tumor suppressor (ING1) that is evolutionarily conserved, encodes several isoforms and is part of a small family of related ING genes. Since then, studies from our lab and in others' have found that ING1 is involved in the regulation of cell growth, apoptosis, cell aging and DNA damage response. Down-regulation or a complete loss of ING1 expression is observed in a variety of cancers, and certain tumor types also show ING1 mislocalization. ING1 has been shown to translocate to the cell nucleolus and interact with PCNA in a stress (UV light) induced fashion. The nucleolar translocation of ING1, plus its PCNA interacting protein (PIP) domain are both necessary for the ability of ING1 to induce apoptosis after lethal amounts of DNA damage. Here we report that a small pool of ING1 gets translocated to the cytoplasm after cells are subjected to sub-lethal amounts of DNA damage. This change in localization of ING1 from nucleus to the cytoplasm after UV light and γ-irradiation induced DNA damage, was confirmed by immunofluorescence analysis as well as by cell fractionation and Western-blotting. Further experiments are underway that would help us better understand the cellular dynamics of ING1 localization after DNA damage.

Number	Name	Title/Abstract
3	Marie Eve Brault	Mechanisms implicated in the sensitization of cancer cells to chemo therapeutics drugs by expression of a mutant temomerase RNA template.

In contrast to most somatic cells, telomerase activity is detected in 85% of cancer cells. The remaining 15% of cancer cells utilize an alternative mechanism of telomere maintenance (ALT-alternative lengthening of telomeres). The differential expression of telomerase in cancer cells makes it an attractive target for anti-cancer therapy. Thus, most anti-telomerase strategies developed to date focus on the inhibition of telomerase activity notably by the expression of a dominant-negative catalytically-inactive mutant of hTERT, antisense hTR or by the utilization of various reverse transcriptase inhibitors. However, these anti-telomerase strategies pose several limitations including the extensive telomere shortening required to observe antiproliferative effect, the development of drug resistance and the ineffectiveness of those treatments on ALT cells. An alternative to telomerase inhibition-based therapy consists of targeting the integrity of telomeres rather than the enzyme activity. One of these approaches has been validated by our lab and others and consists of destabilizing telomeres with a mutant human telomerase RNA (hTR) template that dictates synthesis of mutant telomere sequence. The incorporation of mutated repeats at telomeres leads to the sensitization of cells to chemotherapeutic drugs, independently of telomere length and mechanism of telomere maintenance.

Number	Name	Title/Abstract
4	Brett Cleland	Steps in yeast tRNA gene transcription targeted by replication stress signalling

Our lab has generated four different cancer cell lines that express a mutant hTR (MuA-hTR) which allows the synthesis of altered telomeric sequences.1 These cell lines exhibit increased sensitivity to chemotherapeutic drugs such as DNA damaging or cytotoxic agents, manifested by decreased cell proliferation. However, other aspects of the response of these cell lines to drug treatment differ. Interestingly, these cell lines possess different p53 status and average telomere length, which could contribute to the differences observed. We are currently analyzing the mechanisms implicated in the increased sensitivity of these cells to drugs and the requirement for p53 activation in this sensitivity. We hypothesize that the incorporation of mutant hTR sequences disrupts the binding of telomeric proteins on telomeres, affecting telomere integrity and engaging the DNA damage pathway. Consistent with this hypothesis, we found increased 53BP1 and ATM-P foci at the telomeres by immunofluorescence in MuA-hTR expressing cells compared to cells not expressing MuA-hTR. We assessed both senescence and apoptosis as possible mechanisms for the observed decrease in cell proliferation. We did not observe senescence in any of the cell lines. However, treatment with doxorubicin led to altered cell cycle profile and apoptosis in one cell line. We are currently investigating the activation of molecules in both p53-dependent and independent signaling pathways. We are validating the specificity of MuA-hTR telomere destabilization in telomerase-positive cells. Since telomerase is absent or weakly active in primary cells, we hypothesize that expression of a mutant hTR should minimally affect the proliferation of primary cells.

Poster Abstracts

Number	Name	Title/Abstract
5	Dr. Pauline Douglas	Identification of protein phosphatases that interact with DNA-PK: The possible role of PP6 in the DNA damage response. The catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs) is a large polypeptide of over 4000 amino acids in length. DNA-PKcs plays a critical role in the repair of DNA double strand breaks (DSBs) produced by ionizing radiation (IR) and certain chemotherapeutic drugs. Studies from several laboratories including our own have shown that the protein kinase activity of DNA-PK is required for DSB repair in vivo. Protein phosphorylation by protein kinases and protein dephosphorylation by protein phosphatases is the most common mechanism for regulating a plethora cellular processes. Protein phosphatases fall into major families based on specificity, different amino acids sequences, structures and catalytic mechanisms. The protein Ser/Thr phosphatase PPP family comprises the type 1 (PP1) and type 2A (PP2A, PP2B, PP4, PP5, PP6 and PP7) protein phosphatases that are conserved through evolution. Members of the type 2A group of phosphatases (PP2A, PP4 and PP6) are the most closely related in sequence (50% identity at the amino acid level) and are sensitive to inhibition by low doses of okadaic acid (OA), a property that distinguishes them from type 1 protein phosphatases. In 2000 the Lees-Miller group carried out studies with OA and suggested that DNA-PK was regulated in vitro and in vivo by a type 2A (PP2A) or type 2A-like protein phosphatase (PP2A, PP4 or PP6) (Douglas et al, J. Biol. Chem. 2001). Here, we have examined whether PP2A or PP2A-like protein phosphatases interact with DNA-PKcs. We show that the catalytic subunit of PP6 interacts directly with DNA-PKcs in vitro and in vivo. The role of PP6 in the regulation of DNA-PK and the DNA damage response is currently under investigation.
6	Charlene Downey	Osteochondroprogenitor-specific deletion of a tRNA required for normal selenoprotein function leads to abnormal skeletal development Selenium, an essential dietary micronutrient, is a key co-factor for a group of molecules known as selenoproteins, which number 24 in the mouse genome. Most selenoproteins function as antioxidants, but they have also been shown to have other important enzymatic functions, such as conversion of thyroid hormone T4 to the T3 form. Selenium deficiency has been associated with an increased incidence of cancer, cardiovascular disease, asthma, and a syndrome known as Kashin-Beck osteoarthropathy. The latter, seen in western China, Tibet and Siberia, is characterized by abnormal endo-chondral bone growth and appears to be due, at least in part, to a lack of soil (and hence plant and water) selenium. Mice with a knock-out mice of the tRNA responsible for selenocysteine incorporation into selenoproteins show embryonic lethality, hence studies involving deletion of this tRNA must be done via a conditional mutagenesis approach. To explore the in vivo importance of normal selenoprotein function in the developing skeleton, mice with floxed selenocysteine tRNA (SectRNA) alleles were interbred with mice expressing the Cre recombinase under the control of the chondrocyte-specfic type II collagen (Col2a1) gene promoter. This would be predicted to greatly diminish seleno-protein function in the developing skeleton and

Number	Name	Title/Abstract
		other cartilagenous tissues. The knockout mice, originally similar in size to the controls, showed growth retardation with under-developed ears, shortened snouts, and evidence of respiratory distress terminally, such that they required euthanasia at ~5 weeks of age. The respiratory distress, with marked indrawing of the ribs cage, appears to be due to tracheal cartilage hypoplasia and/or tracheomalacia. We have used microcomputed tomography, histology and Immunohistochemistry to further characterize the observed phenotype. By studying the effects of Sec tRNA deficiency on the skeletal system, we can not only begin to investigate the role of specific selenoproteins, but also possibly discover a role for selenium supplementation in the preservation of bone and maintaining the integrity of cartilage.
7	Stephanie Hiebert	Phophorylation of Artemis
8	Michael Ho	Development of an in vitro and in vivo activity assay for the Histone Methyltransferases, Ezh2 and Smyd3. Epigenetic mechanisms allow cells to stably inherit cellular properties in somatic cell divisions without affecting the DNA code. We are interested in two histone methyltransferases that are involved in the epigenetic pathway, EZH2 and SMYD3. EZH2 and SMYD3 have been found to be overexpressed in the early stages of many types of cancer. Studies have shown that siRNA depletion of EZH2 and SMYD3 can induce apoptosis in hepatocellular carcinoma cells. In light of these promising data, the goal of my project is to develop an assay to enable the screening for inhibitors of EZH2 and SMYD3. The assay will be adapted for high throughput screening of a combinatorial drug library. It is designed to monitor the histone methyltransferase activity by in vivo or in vitro Fluorescence Resonance Energy Transfer (FRET). I have generated recombinant proteins for EZH2 and SMYD3 and will use these purified proteins to make antibodies, which will be used to affinity purify the EZH2 and SMYD3 functional complexes in vivo. The recombinant proteins will also be the basis of the in vitro assay.
9	Chris Knight	Characterization of the BRCT domain in DNA polymerase X family-mediated DNA repair Double strand breaks (DSBs) are a form of DNA damage that can be repaired either by homologous recombination (HR) or by non-homologous end joining (NHEJ). A number of proteins known to be involved in these repair pathways, such as BRCA1, 53BP1 and DNA Ligase IV, contain at least one BRCA1 Carboxyl Terminal (BRCT) domain. This domain has been shown to mediate both protein-protein and protein-DNA interactions, as well as at times, to have an affinity for phospho-peptide motifs. Here, we characterize the interaction between the BRCT domains of DNA Ligase IV, and DNA polymerase λ (Pol λ) with members of the NHEJ machinery. GST tagged BRCT fragments were developed from these proteins and GST pull down assays were performed in both crude extracts and with purified proteins. We were able to observe the previously characterized interaction between XRCC4 and the tandem BRCT domain of DNA Ligase IV. Using this as a basis for our experiments, other possible

Number	Name	Title/Abstract
		BRCT mediated interactions with NHEJ factors were investigated. Here, we show that the BRCT domain of Pol λ interacts with Ku70/80, but this is dependent upon the presence of DNA.
11	Dr. Artee Luchman	Age-related effects of deleting the Pten tumour suppressor in prostate epithelium: towards a physiologically-appropriate mouse model of human prostate cancer There is a paucity of murine models able to mimic human prostate cancer. In existing models, progression through the different cancer grades can be greatly accelerated with disease occurring shortly after puberty. This timing contrasts with the human situation, where prostate cancer progresses very gradually, becoming overt only with advanced age. To better mimic the delayed latencies of the human disease, we employed targeted transgenesis to generate a mouse line which permits external control over the timing of gene deletions. This system was used to investigate the effects of deleting the Pten tumour suppressor, in both the developing and mature glands. We found that despite producing prominent activation of pro-proliferative pathways, Pten excisions in the fully developed gland led to a more gradual development of a range of premalignant lesions; a progression more reflective of human prostate cancer. Our line is hence ideally suited to assaying factors for their ability to accelerate tumour progression and will prove useful for studying the consequences of gene excisions in adult males. This system will also allow us to investigate the effects of aging on the oncogenic process and will be useful for evaluating the impact that lengthy exposures to special diets, trace element and vitamin deficiencies, as well as putative prostate carcinogens or tumour promoters may have on the progression of tumorigenesis.
12	Brandi Mahaney	Characterization of the intrinsic DNA binding affinity of XLF Double strand DNA breaks (DSBs) are one of the most detrimental DNA lesions in the cell. In mammalian cells these DSBs can be repaired by the non homologous end joining pathway which involves several core proteins including Ku, DNA-PKcs and the XRCC4-ligase IV complex, as well as several accessory factors for efficient repair. XRCC4-like factor, XLF, is the most recently discovered non-homologous end-joining factor. The exact function of XLF has not yet been determined, however recently published crystal structures show that it is structurally similar to XRCC4. Here we show using electrophoretic mobility shift assays that XLF binds to a DNA in a length-dependent manner. There does not appear to be a significant difference in the affinity of XLF for DNA containing an overhanging end compared to blunt ended DNA and multiple XLF molecules appear to bind to a single 300 bp DNA molecule suggesting the XLF-DNA interaction is not limited to DNA ends. Alternatively, XLF may be capable of forming DNA-bound multimers. Significantly, we find that the C-terminal region of XLF has even greater

Number	Name	Title/Abstract
		affinity for dsDNA than the full-length protein which could suggest that the predicted stalk region of XLF inhibits DNA binding in the intact protein. Finally, our lab has recently identified DNA-PK phosphorylation sites in the C-terminus of XLF, however, phosphorylation of XLF at these sites does not significantly affect binding of DNA by the C-terminus. These data provide the first clear demonstration of a DNA binding function for XLF and suggest a novel DNA binding mechanism for XLF.
13	Dr. Rajam Mani	Biophysical Characterization of Human XRCC4 and its interaction and activation of Human Polynucleotide Kinase Nonhomologous end joining (NHEJ) is the major DNA double-strand break repair pathway in mammalian cells. NHEJ involves the co-ordinated assembly of several protein factors including DNA-PK, the XRCC4-DNA ligase 1V complex. Recent studies have also shown a role for polynucleotide kinase (PNK) in DSB repair and phosphorylated XRCC4 (Thr 233) has been shown to bind tightly to the forkhead associated (FHA) domain of PNK. We are examining the interaction between XRCC4 and PNK using a variety of bio-physical techniques. Analytical ultracentrifugation revealed that XRCC4 (non-phosphorylated form) exists in equilibrium between a dimeric and tetrameric state. However, when XRCC4 and PNK were mixed in a 1:1 molar ratio, the sedimentation data fitted well for a single entity with a calculated Mr of 90000 suggesting a 1:1 complex of XRCC4 and PNK. Binding of non-phosphorylated XRCC4 to PNK was confirmed by far UV-CD, fluorescence quenching and co-immunoprecipitation. When we analyzed the interaction of the FHA domain of PNK to the phosphorylated and the non-phosphorylated XRCC4, we observed that non-phosphorylated XRCC4 failed to bind to the FHA domain while phosphorylated XRCC4 bound tightly (Kd ~ 5 nM). Taken together these observations suggest that there are two modes of interaction between XRCC4 and PNK: a high affinity FHA and phospho-T233 mediated interaction and another lower affinity interaction that is not dependent on XRCC4 phosphorylation.
14	Laura Minard	Genetic and functional interactions between histone chaperone Asf1 and Snf2, the catalytic subunit of the SWI/SNF chromatin remodelling complex Asf1 is a conserved histone chaperone that functions to deliver histones H3 and H4 to DNA, or to remove these histones from DNA. As such, Asf1 plays a key role in the regulation of chromatin processes, including DNA replication, transcription and the DNA damage response. The molecular mechanisms underlying the functions of Asf1 in chromatin regulation however are poorly understood. Here we present evidence that yeast Asf1 interacts genetically and functionally with the SWI/SNF chromatin remodelling complex, which uses the energy from ATP hydrolysis to alter the contacts of the DNA with the histones. In a synthetic genetic array analysis we uncovered an interaction between ASF1 and SNF2, the gene encoding the catalytic subunit of the SWI/SNF complex. This interaction was confirmed by random spore analysis. asf1Δ snf2Δ double mutants are slow-growing, and are more sensitive than

Number	Name	Title/Abstract
		the single mutants to agents that cause DNA damage or replication stress. asf1Δ snf2Δ double mutants also have more pronounced checkpoint and cell cycle defects than the asf1Δ single mutant; they fail to fully arrest in S phase in response to hydroxyurea (HU), a replication inhibitor, and show defects in recovering from prolonged exposure to HU. asf1Δ snf2Δ cells are capable of activating the DNA damage response, but they do so slowly in comparison to the asf1Δ single mutant. Thus, asf1Δ snf2Δ cells have phenotypes consistent with partially overlapping functions in cell cycle and checkpoint control. Northern blotting analysis revealed that the induction of DNA damage response genes in response to HU is more severely blocked in asf1Δ snf2Δ double mutants than either single mutant, suggesting that Asf1 and Snf2 may work together in the context of transcription. Consistent with a direct role for Asf1 in transcription, Asf1 was detected by chromatin immunoprecipitation at the promoters of all genes analyzed. Strikingly, following treatment with HU, Asf1 occupancy increases at all loci tested, including genes that are repressed and derepressed by HU. We propose a model in which Asf1 is further recruited to DNA following replication stress to participate in chromatin assembly/disassembly, possibly in concert with SWI/SNF at promoters.
15	Vesna Nguyen	DNA damage checkpoint signalling to the tRNA genes In budding yeast, transcriptionally active tRNA genes are refractory to repair of single strand DNA lesions, and can interfere with replication fork movement in a way that may promote chromosome breakage. Because the high rate of tRNA transcription during proliferation might increase the incidence of detrimental genetic changes at tRNA genes, it could be advantageous for cells to use DNA damage checkpoint signalling to repress transcription under conditions of genotoxic stress. We have explored the idea that checkpoint proteins control tRNA gene transcription using a candidate gene approach. Here we show that repression of the tRNA genes in cells treated with the DNA alkylating agent methyl methanesulfonate (MMS) requires the conserved checkpoint signalling kinase Rad53. Because Rad53 plays a central role in the response to double strand breaks (DSBs) and MMS was reported to directly induce DSBs, we tested if ionizing radiation or a radiomimetic drug trigger tRNA gene repression. Surprisingly, they do not. We therefore turned our attention to the possibility that repression by MMS is principally a response to replication interference (recent studies argue that DNA damage checkpoint engagement in MMS-treated cells is mainly due to replication stress rather than direct generation of DSBs). Consistent with this possibility, we observed strong repression of tRNA genes in cells treated with hydroxyurea (HU), which interferes with replication because it causes depletion of deoxyribonucleoside triphosphates. This downregulation partly involves Rad53 and Maf1, a universal repressor of the tRNA transcriptional machinery in yeast. Maf1 likely functions in this repression as a transcriptional regulator, since activation of Rad53 by HU is normal in the absence of Maf1. In addition to finding that Rad53 is important for repression of the tRNA genes under conditions of genotoxic stress, we made the unexpected

Number	Name	Title/Abstract
		discovery that Rad53 represses tRNA gene transcription in normally cycling cells. We therefore are testing the hypothesis that DNA structure checkpoint control of the tRNA genes enhances fitness because it directly couples the detection of replication stress to inhibition of the capacity of tRNA genes to function as barriers to replication.
16	Dr. Linda Sandercock	O6-Methyguanine methyltransferase (Mgmt) deficiency alters the in vivo mutational spectrum of tissues exposed to the tobacco carcinogen 4-(mathylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) It has been proposed that O(6)-methylguanine DNA methyltransferase (MGMT) gene silencing in premalignant lesions and cancers of the lung might result in the acquisition of a 'mutator' phenotype. Previously, however, we found that Mgmt(-/-) mouse DNA failed to show an increase in spontaneous mutations. We thus hypothesized that only during exposure to specific environmental carcinogens would the consequences of MGMT deficiency become evident. Metabolism of the tobacco-derived nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) generates alkylating species that can react with the O(6) position of deoxyguanine, thereby yielding substrates for MGMT-mediated repair. To investigate how MGMT might regulate the mutational effects of NNK, Mgmt (-/-) mice were crossed with a lacI-based transgenic reporter line (Big Blue) thus enabling an assessment of the in vivo mutagenic effects of this agent. We observed the induction of a complex spectrum of NNK-dependent lacI mutations in both control and Mgmt(-/-) tissues, but only a trend in the mutant frequency increases that could be attributed to MGMT deficiency. The mutational spectra of NNK-treated Mgmt(-/-) lungs revealed an increase in the absolute number of G:C to A:T changes accompanied by a shift in these from CpG to GpG sites, consistent with an S(N)1 alkylation mechanism. In keeping with the high levels of MGMT expressed in the liver, more pronounced mutagenic effects and greater differences in O(6) position of deoxyguanosine adduct levels following NNK were observed in Mgmt(-/-) versus wild-type mice. Extrapolating to humans, MGMT-deficient cells would likely exhibit an increased mutational burden, but only following exposures to specific environmental mutagens such as NNK.

Number	Name	Title/Abstract
17	Sitar Shah	A novel role for the chromatin modulator ING3 in C. elegans apoptosis The INhibitor of Growth (ING) family proteins are involved in multiple cellular processes such as growth regulation, DNA repair, and apoptosis. ING proteins are activated by stresses, including ionizing radiation, leading to the activation of p53 and consequently apoptosis. ING proteins in mammals and yeast have recently been shown to read the histone code in a methylation-sensitive manner, targeting histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes to chromatin, which subsequently regulate gene expression.Loss or downregulation of ING protein function is observed frequently in several tumor types, thus warranting their classification as type II tumor suppressors. Five ING genes have been discovered in mammals and three are found in Caenorhabditis elegans (C. elegans). Following UV irradiation, ING3 expression is rapidly induced in melanoma cells, promoting apoptosis. ING proteins in mammals share various overlapping functions, making the interpretation of individual roles challenging due to redundancy. Here we identify and characterize C. elegans ing-3, the closest ortholog for mammalian ING3. ING3 co-localizes with chromatin in C. elegans embryos, germ line and somatic cells. Both ing-3RNAi transfected worms and ing-3 mutant strains demonstrate that the gene functions in the same pathway as the C. elegans p53 homolog, cep-1, to induce germ cell apoptosis in response to ionizing radiation. This study not only demonstrates the importance of C. elegans ing-3 in the DNA damage response pathway, but it is also the first to implicate ING proteins in C. elegans germline and embryonic development.
18	Reem Skeik	Recombination bad news: the need to prevent recombination at stalled replication forks We engineered a novel DNA polymerase delta (DNAP δ) in the budding yeast Sacharomyces cerevisiae that is sensitive to the antiviral drug phosphonoacetic acid (PAA).1 We are using the drug-sensitive DNAP δ to study genes involved in the post replication repair (PRR) pathway. PRR or the Rad6 pathway has two components. One component uses error-prone, translesion synthesis (TLS) DNA polymerases and the second uses error-free homologous recombination. Differential use of the two pathways depends on the ubiquitination of PCNA. For TLS, PCNA is mono-ubiquitinated by the Rad6 ubiqutin conjugating enzyme and Rad18, the ubiquitin ligase responsible for the ubiquitination of PCNA. PCNA is poly-ubiquitinated for the recombination pathway. The rad6 pol3-612 double mutant is very sensitive to PAA, which suggests that PCNA ubiquitination is important for the recovery of stalled replication forks produced by PAA inhibition of the L612M-DNA pol delta. However, instead of PAA-sensitivity, the rad18 pol3-612 double mutant was less sensitive to PAA than the singly mutant pol3-612 strain. We are now determining how the absence of Rad18 function rescues PAA-sensitivity of the pol3-612 strain. Our working model is that there is competition for PCNA at stalled replication forks. While mono-

Number	Name	Title/Abstract
		ubiquitination of PCNA directs TLS DNA polymerases to replication forks, DNA damage is really not a problem for stalled replication forks produced by PAA. However, pol3-612 cells are very sensitive to PAA in the absence of the Srs2 helicase (A. Sonpar). Srs2 also needs PCNA for function, but Srs2 needs SUMO-modified PCNA instead of Ub-PCNA. The reduced PAA-sensitivity of the rad18 pol3-612 strain suggests that increased SUMO-PCNA and as a consequence increased Srs2 activity is produced in the absence of Rad18. If true, then there is a need for more Srs2 activity at stalled replication forks produced by PAA than is available normally. Since the Srs2 helicase is an anti-recombinase, we propose that stalled replication forks produced by PAA are prone to the formation of deleterious recombination products. These results, however, do not explain the PAA-sensitivity of the rad6 pol3-612 strain; preliminary observations will be presented.

Number	Name	Title/Abstract
20	Heather Smith	**Determining the binding partners of the Novel Conserved Region (NCR) of ING1** The ING family of tumour supressors are involved in apoptosis and cell senescence through epigenetic regulation of gene expression by physical interaction with histone acetyltransferase (HAT) and histone deacetylase (HDAC). INGs (1-5) and their splicing isoforms share a conserved plant homeodomain zinc finger motif and a nuclear localization sequence. Bioinformatic analysis of all known ING proteins identified a ~50 amino acid novel conserved region (NCR) in all ING family members. Our hypothesis is that this novel region may play a role in chromatin remodeling and the subsequent regulation of gene expression. The Basic Local Alignment Search Tool (BLAST) was used to determine whether the NCR defines a new protein motif and if it is ING-specific. There were 140 matches of the query sequence, most of which were members of the ING family in human and mouse, and in their distant homologs in Xenopus laevis and Drosphila melanogaster. Multiple sequence alignments were done looking for similarity with known chromatin remodeling proteins and it was found that NCR is unique to the ING family proteins. Potential binding partners with NCR are being investigated. This study has the potential to clarify the biochemical mechanisms used by the class II tumour suppressor ING1 in proliferation, DNA repair, apoptosis, and senescence.
21	Mohamed Soliman	**The tumor suppressor ING1 mediates cellular senescence through chromatin remodeling** The INhibitor of Growth 1(ING1) is type II tumor suppressor proteins involved in the regulation of cell growth, apoptosis and cell aging. ING1 mediate their diverse biological effects through altering chromatin structure by binding core histone and targeting associated HAT (Histone Acetyltransferase) and HDAC (Histone Deacetylase) activity, subsequently affecting transcriptional regulation. Decreased ING1 expression and mislocalization have been confirmed in several primary human cancers and established cancer cell lines. So far, three splicing variants of ING1 have been identified, ING1a, ING1b and ING1c. ING1a and ING1b proteins are the two major splicing isoforms of ING1 expressed in mammalian cells. Since ING1b is expressed at higher levels, most studies have focused upon the role of ING1b in inducing apoptosis in both normal and cancer cells, in p53 dependent and independent manners. We found that, as human diploid fibroblasts enter into a state of replicative senescence, the relative amounts of the different isoforms of the ING1 gene are altered dramatically, with the ratio between ING1a and ING1b mRNA's being altered by 30-fold. High levels of ING1a mRNA accumulate in senescent human diploid fibroblasts, whereas young cells show preferential expression of ING1b mRNA. Consistent with this idea, our recent experiments suggest that ING1a promotes and/or induces cell cycle arrest and formation of senescence-associated heterochromatic foci that are indicative of a senescent phenotype. These observations suggest that different ING1 isoforms might perform their antitumor functions through distinct chromatin-remodeling mechanisms, with ING1b promoting apoptosis

Number	Name	Title/Abstract
		and ING1a promoting senescence. Identification of ING1a as a new senescence-marker may aid development of high-throughput screening systems for agents that can induce senescence in tumor cells.
22	Ashlesha Sonpar	A Novel Role for Helicases at Replication Forks DNA helicases are important enzymes in all living organisms; they unwind DNA for replication, repair and recombination. Defects in helicase function can lead to genomic instability. Most notably, RecQ helicases have recently received much attention because of their role in regulating recombination and the well known correlation between mutations in these genes in humans and premature aging and cancer. However, there is another helicase family – the UvrD family that also functions in replication, repair, and recombination. While the yeast (Saccharomyces cerevisiae) UvrD-type helicase Srs2 is considered to be functionally redundant with the Sgs1 RecQ helicase, we have found an essential role for Srs2 at stalled replication forks. We use a mutant DNA polymerase delta (L612M-DNA pol d), which is sensitive to the antiviral drug phosphonoacetic acid (PAA)1, to produce stalled replication forks. PAA inhibits replication by the L612M-DNA pol d, but PAA-sensitivity is greatly increased in the absence of the Srs2 helicase. We are now characterizing the Srs2 pathway.
23	Dr. Nasser Tahbaz	The role of kinase and phosphatase activities of hPNK in response to DNA damage induced by different genotoxic agents Human polunucleotide kinase (hPNK) is an enzyme with independent DNA kinase and phosphatase activities. hPNK is a key enzyme to ensure the correct chemical configuration at the DNA ends, 5'-phosphate and 3'-hydroxyl, prior to DNA repair. Previous data from the Weinfeld lab indicated that RNAi-induced inhibition of hPNK expression renders cells more sensitive to radiation and chemotherapeutic agents that generate DNA breaks with modified ends. We asked whether a particular enzymatic activity of hPNK, or both, is/are required for repair of DNA damage induced by a specific agent. We have generated cancer cell lines in which the endogenous hPNK has been replaced by the hPNK carrying only the kinase or phosphatase activity. These cells will be tested for their sensitivity to different DNA damaging agents and their capacity for the repair of single and double-strand breaks.

Number	Name	Title/Abstract
24	Anna Urbanska-Edmond	Prolactin and the DNA damage response in breast cancer cells

Prolactin is a peptide hormone with four main functions in the mammary gland: proliferation, survival, differentiation and motility. It plays an important role in breast cancer as prolactin can trigger the growth and motility of human breast cancer cells (Clevenger et al, 2003) and increased serum levels are associated with increased risk of breast cancer (Tworoger & Hankinson, 2008). There are at least three different signaling pathways that transduce from prolactin binding to its receptor. Currently, our research is focused on the Jak/Stat pathway transduced by prolactin binding to its receptor, which is associated with mammary gland differentiation, survival and proliferation (Clevenger et al, 2003). It has been previously discovered in our lab that the activation of the Jak/Stat pathway results in the transcription of heat shock protein 90 alpha (Hsp90 alpha), a master cancer chaperone (McClellan et al, 2007). Our preliminary evidence suggests that Hsp90 alpha acts as a molecular chaperone for ataxia talengiectasia mutated (ATM) (Shemanko, unpublished results), a protein critical for double stranded DNA break repair (Tomimatsu et al, 2007). Therefore, prolactin up regulates hsp90 alpha through the Jak/Stat pathway, which acts as a molecular chaperone and stabilizes ATM. We hypothesize that breast cancer cells undergoing radiation might be more resistant and have an increased chance of survival in the presence of prolactin. Prolactin is linked to the DNA damage response pathway via Hsp90 alpha, thus cells repairing their DNA may have an advantage with increased prolactin levels. This study investigates the effect of prolactin and 17-N-allylamino-17-demethoxy geldanamycin (17-AAG), a specific inhibitor of Hsp90 (alpha and beta), on the survival of human breast cancer cells that have undergone radiation treatment. The approach we have used to study cell survival is a clonogenic assay. SKBR3 human breast cancer cells were pre-treated with prolactin or 17-AAG for 24 hours and irradiated at various dosages of gamma radiation. The cells were then cultured for 12 days and survival was measured quantitatively by the number of surviving cells that were able to form colonies that contained more than 50 cells.

Our clonogenic assay results show that with increased radiation levels, cell survival decreases. When SKBR3 are treated with 17-AAG prior and during irradiation, cells are sensitized to radiation treatment at 6 Gy ($p = 0.002$). Cells treated with prolactin prior and during irradiation and during the recovery period show an increase in survival at 2 Gy. SKBR3 treated with 17-AAG prior and during irradiation with prolactin included for the recovery period show an increase in survival at 6Gy ($p = 0.0004$) and 8Gy ($p = 0.023$). These results indicate that patients with increased prolactin levels may experience resistance to conventional cancer therapy. The combination of 17-AAG and radiation might improve treatment of breast cancer for patients with high serum prolactin levels and/or increased expression of Hsp90 alpha in the breast tumor.

Number	Name	Title/Abstract
25	Larissa Vos	Reduced mitotic checkpoint proteim hZw10 on the kinetochores of aneuploid breast cancer cells. Our lab is interested in mitosis and how it is regulated. Mitosis is the process by which a cell equally distributes its chromosomes into two daughter cells. Accurate chromosome segregation depends upon the bipolar attachment of each kinetochore to the spindle microtubules (MT) and alignment at the metaphase plate. If there is even a single unaligned chromosome, the mitotic checkpoint will delay anaphase onset until all chromosomes are attached and aligned properly. The loss of the mitotic checkpoint results in chromosome missegregation and may result in cell death or aneuploidy and the evolution of cancerous cells. Aneuploidy is an abnormal number of chromosomes in the cell and is a common characteristic of solid tumors. The kinetochore is a proteinaceous structure that forms on the centromere specifically during mitosis and is responsible for MT attachment and the mitotic checkpoint. Human Zw10 (Zeste White 10) is a kinetochore protein that form an evolutionarily conserved complex with the kinetochore protein hRod (Roughdeal). This complex recruits dynein/dynactin to the kinetochore and is an essential component of the mitotic checkpoint. We have found that the kinetochore residency of hZw10 is reduced is a subset of breast cancer cell lines compared to nontumorigenic and cancer cell lines with a robust mitotic checkpoint. We are investigating whether the reduced kinetochore occupancy of hZw10 could lead to a weakened mitotic checkpoint and the development of aneuploidy.
26	Chris Williamson	Inhibition of PARP-1 is cytotoxic in ATM deficient Mantle Cell Lymphoma cells Mantle Cell Lymphoma (MCL) is an extremely aggressive B-cell malignancy. Existing treatment provides an average patient survival of only 3 years. Up to half of all MCL tumors contain a disruption of the Ataxia-Telangiectasia Mutated (ATM) gene, either through mutation of the coding sequence or loss of the gene locus. ATM is a large serine/threonine protein kinase that is activated in response to DNA double strand breaks. It was recently demonstrated that inhibition of the DNA single strand break repair protein Poly-ADP Ribose Polymerase-1 (PARP-1) is cytotoxic to cells that contain an existing DNA repair defect. Based on these observations, we speculated that ATM-deficient MCL cells might likewise be susceptible to PARP inhibition. In this study, we have characterized ATM status in four MCL cell lines including one, Granta-519, with a known defect in ATM. The Granta-519 cell line was shown to display cellular characteristics similar to those of known ATM defective cell lines and was significantly sensitive to PARP-1 inhibition. In addition, reduction of ATM protein levels with shRNA increased the sensitivity to PJ34 in an otherwise ATM proficient MCL cell line. Based on these results, we speculate that inhibition of PARP-1 could have therapeutic potential for the management of MCL.

Number	Name	Title/Abstract
27	Dr. Yaping Yu	DNA-PK phosphorylation sites in XLF are not required for repair of DNA double strand breaks

Nonhomologous end joining (NHEJ) is one of the major pathways for the repair of DNA double strand breaks (DSBs) in human cells. Proteins involved in NHEJ include the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), Ku70, Ku80, XRCC4, DNA-ligase IV as well as Artemis, DNA polymerases μ and λ and polynucleotide kinase. Recent studies have identified an additional participant, XLF, for XRCC4-like factor (also called Cernunnos). XLF interacts with the XRCC4-DNA ligase IV complex and stimulates its activity *in vitro*, however, its precise role in the DNA damage response is not fully understood. The protein kinase activity of DNAPKcs is required for NHEJ and DSB repair *in vivo*; therefore, we asked whether XLF might be a physiological target of DNA-PK. Here, we have identified two major *in vitro* DNA-PK phosphorylation sites in XLF (serines 245 and 251), which are located in the C-terminal region of XLF. Using metabolic labeling and mass spectrometry, we show that these sites also represent the major phosphorylation sites in XLF *in vivo*. Using phosphospecific antibodies we show that serine 245 is phosphorylated *in vivo* by DNA-PK and that serine 251 is phosphorylated by Ataxia-Telangiectasia Mutated (ATM). However, phosphorylation of XLF did not affect the ability of XLF to interact with DNA *in vitro*, its recruitment to laser induced DSBs *in vivo* or the rate of DSB repair (as judged by loss of γ-H2AX foci) *in vivo*. Finally, XLF in which six of the identified phosphorylation sites had been converted to alanine complemented the repair defect in XLF-deficient 2BN *in vivo*. Therefore, we conclude that phosphorylation of XLF at these sites is unlikely to be required for repair of IR-induced DSBs *in vivo*.

Number	Name	Title/Abstract
28	Ranran Zhang	A yeast DNA pol delta mutant sensitive to hydroxyurea

Many DNA polymerases have both polymerizing and 3'-5' exonucleolytic proofreading activities. The balance between primer extension and proofreading determines the polymerase activity and the fidelity of DNA replication. Bacteriophage T4 DNA pol mutants that have reduced proofreading activity replicate DNA with low fidelity (mutator phenotype) and many are sensitive to the antiviral drug phosphonoacetic acid (PAA) (1). Second-site mutations that suppress PAA sensitivity often produce new phenotypes: sensitivity to a decreased dGTP pool and increased replication fidelity (antimutator phenotype) (2). In the budding yeast Saccharomyces cerevisiae, a DNA pol d mutant pol3-V592G was identified in a genetic screen for hydroxyurea-sensitive DNA pol mutants. Hydoroxyurea, or HU, inhibits ribonucleotide reductase so that dNTP pools are decreased. The V592G amino acid substitution was also identified in a genetic selection for suppressors of the PAA sensitivity of the pol3-L612M strain, which encodes a mutant DNA pol d that is PAA sensitive and replicates DNA with low fidelity, as observed for the analogous phage T4 L412M-DNA pol (3). HU sensitivity of the pol3-V592G mutant is also suppressed by the second-site L612M substitution. Because of similarities between the T4 L412M-DNA pol and yeast L612M-DNA pol d, we expected suppressors of PAA sensitivity of the

Number	Name	Title/Abstract
		yeast L612M-DNA pol d would confer an antimutator phenotype. Surprisingly, replication fidelity tests showed that pol3-V592G strain has a mutator phenotype. We demonstrated that the mutator phenotype of the pol3-V592G mutant is not due to translesion DNA polymerases. We are now determining the molecular base of the mutator phenotype.
29	Ran Zhuo	Genetic analysis of cellular responses to caffeine

Caffeine is the most widely used psychoactive drug in the world, praised as a neurological stimulant. Caffeine also exhibits other pharmacological effects, including sensitizing cells to irradiation and genotoxic drugs apparently by overriding cell cycle checkpoint responses, promoting apoptosis and altering intracellular calcium levels. Because of these other effects, caffeine has become a very useful experimental tool as well as a potential adjuvant for cancer chemotherapy. The mechanisms underlying these diverse actions of caffeine have not been well elucidated in vivo. Therefore, it is important to understand the mechanisms underlying the pharmacological activities of caffeine.

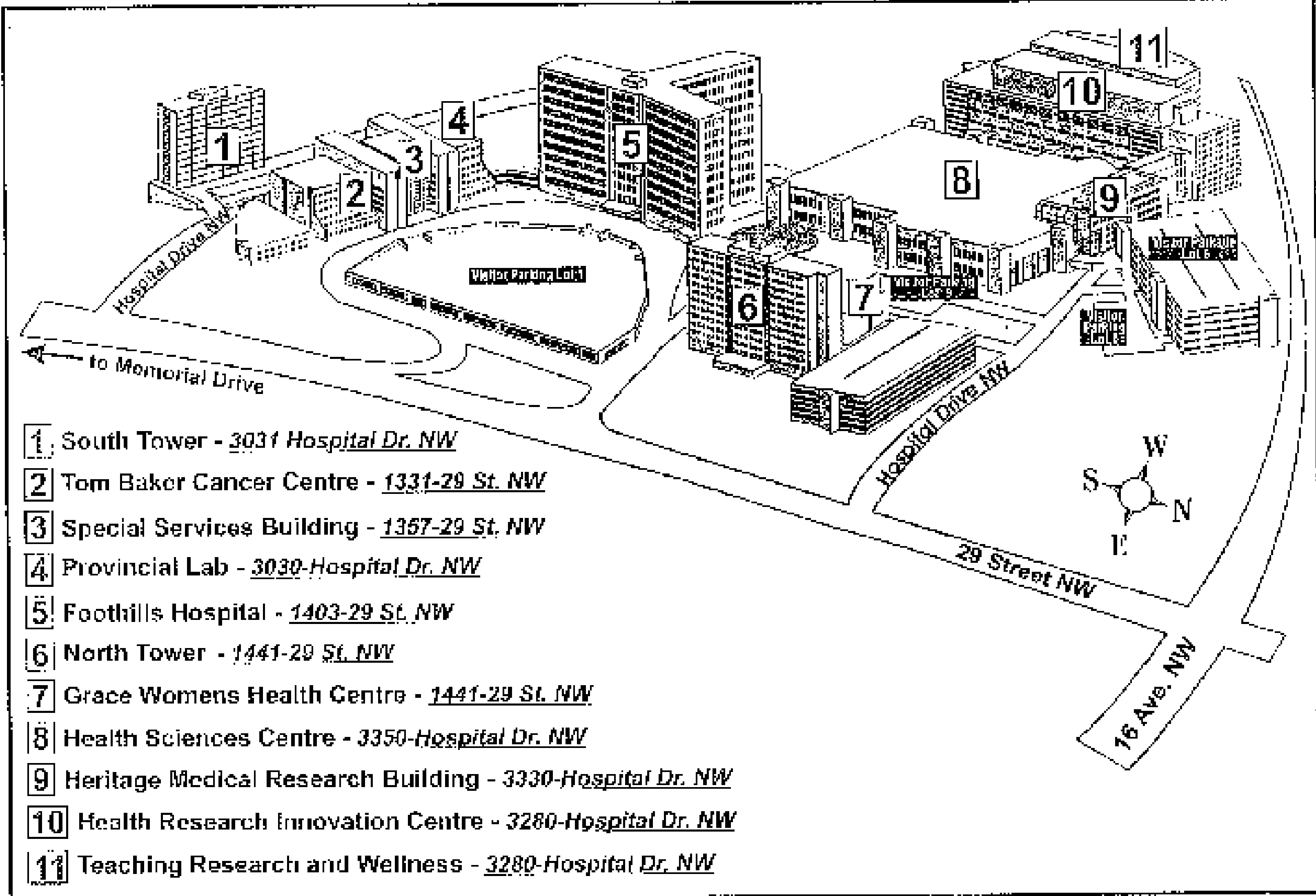
Previously, we described a novel genetic screening strategy to identify conditional mutants that were sensitive to larval ingestion of genotoxic compounds, specifically caffeine and hydroxyurea (Silva *et al*., 2007). An improved caffeine screen was conducted on chromosome arm 3R that identified two new loci and also 6 new alleles of a previously identified gene *jnj* (*java no jive*).One of the two new loci was termed *sst* (*sleepless in Seattle*) and the other *double double trouble* (*ddt*). The new screen was found to be time efficient and more effective in reducing the number of false positives. In order to identify the gene associated with these mutants, eye-specific RNA interference of the candidate genes in those regions were utilized to determine if any of the "knockdowns" were caffeine sensitive. RNAi of a gene *CG13605* in the *jnj* region reproduced the caffeine sensitive phenotype of *jnj*, suggesting it to be the right gene. *CG 13605* is localized cytologically to 95E1 and is suspected to have the molecular function of protein binding and zinc ion binding. It is thought to interact with CG11132 which is a protein involved in negative regulation of transcripts, phagocytosis and engulfment. CG11132 colocalizes with the NuA4 histone acetyltransferase complex which is implicated in double-strand DNA break repair. Sequencing of *CG13605* in the *jnj* hemizygote and homozygote mutants is currently underway to confirm our tentative identification of CG13605 as *jnj* and gain more insight into the function of the *jnj*$^{+}$ gene.

Number	Name	Title/Abstract
30	Angela Zolner	Phosphorylation of polynucleotide kinase

DNA double strand breaks (DSBs) are the most deleterious type of DNA damage. DSB can be caused by both endogenous and exogenous agents, such as reactive oxygen species and ionizing radiation (IR). The phosphatidyl inositol-3 kinase-like kinase (PIKK) family of serine threonine (S/T) protein kinases, Ataxia Telangiectasia Mutated (ATM) and the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), play major roles in mediating the cellular response to DSBs. ATM is primarily involved in signal transduction cascades that initiate cell cycle arrest, regulate DNA DSB repair, and, if necessary, cell death; whereas DNA-PKcs is required for non-homologous end-joining (NHEJ), which is the primary pathway for DSB repair in human cells. Both ATM and DNA-PKcs phosphorylate their substrates on SQ or TQ sites. Polynucleotide kinase (PNK) has 5'-DNA kinase and 3'-DNA phosphatase activities, and has been implicated in NHEJ as well as other DNA repair pathways. Here we have examined whether PNK is phosphorylated in response to DNA damage by the PIKK family of protein kinases. We show that PNK is phosphorylated by DNA-PK in vitro at S114 (an SQ site). Using liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS), we have identified S114 and S126 (also an SQ site) as IR-inducible phosphorylation sites in PNK. Phosphospecific antibodies have been generated to both sites. Using highly selective inhibitors of ATM and DNA-PK (generously provided by KuDos Pharmaceuticals Inc.) we show that IR-induced phosphorylation at S114 of PNK is largely ATM dependent. Phosphorylation of S126 is constitutive and DNA-PK dependent. The functional relevance of the phosphorylation events are currently being characterized and preliminary results will be reported.

Maps - Residence Hotel (Cascade Hotel)

Maps - Parking



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