17th Annual
Buffalo DNA Replication and Repair Symposium

Keynote Speaker

Graham C. Walker Ph.D.
American Cancer Society
Research Professor of Biology
Massachusetts Institute of Technology
Department of Biology

June 28-29, 2013
Hauptman-Woodward Medical Research Institute
17th Annual Buffalo DNA Replication & Repair Symposium

We would like to thank the following for making this Symposium possible:

UB Department of Microbiology & Immunology and the UB Department of Biochemistry for their generous financial support.

Roswell Park Cancer Institute for allowing use of their parking facilities at a discounted rate for the Symposium, and for providing catering needs.

Jill Szczesek and the Hauptman-Woodward Medical Research Institute for allowing us to use their facilities for our Symposium.

The Lunch Box for delivering a fantastic lunch.

The Witebsky Center for Microbial Pathogenesis & Immunology for administrative support.

And we thank our invited speaker, Dr. Graham Walker, who traveled to be with us, and all the attendees, especially those who brought their work to share with all of us.

The organizers are extraordinarily grateful to Kathrine Ohms and Shari Wilson at the Witebsky Center/University at Buffalo for their assistance in organizing this year's symposium.

The organizers are also grateful to Laurie Aronberg and Kate Gunnison at Roswell Park for additional administrative assistance.

We also are grateful to Molly Burhans for preparing artwork for the symposium flyer and Program cover.

Symposium co-organizers: Mark Sutton, Bill Burhans, & Tom Melendy
<table>
<thead>
<tr>
<th>Time</th>
<th>Session I – Chair: Patrick Maxwell</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:30 a.m.</td>
<td>Registration &amp; Poster Setup</td>
</tr>
<tr>
<td>9:55 a.m.</td>
<td>Welcome &amp; Opening Remarks – Tom Melendy, UB School of Medicine</td>
</tr>
</tbody>
</table>
| 10:00 a.m. | COORDINATION OF DNA REPLICATION INITIATION AND TRANSCRIPTIONAL REGULATION BY *ESCHERICHIA COLI* BETA SLIDING CLAMP  
Babu , Vignesh M.P. and Mark D. Sutton  
Department of Biochemistry, State University of New York at Buffalo |
| 10:20 a.m. | PROTEASE STALLING GENERATES CRITICAL CLAMP LOADER DIVERSITY  
Vass, Robert H. and Peter Chien  
Molecular and Cellular Biology Graduate Program, Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA 01003 |
| 10:40 a.m. | CONTRIBUTION OF MMR TO DAMAGE INDUCED MUTAGENESIS IN *ESCHERICHIA COLI*  
Baron, Christopher M. and Mark D. Sutton  
Department of Biochemistry, State University of New York at Buffalo |
| 11:00 a.m. | TRAPPING AND VISUALIZING INTERMEDIATE STEPS IN THE MISMATCH REPAIR PATHWAY IN VIVO  
Lenhart, Justin S. 1§, Monica C. Pillon2§, Alba Guarné2 and Lyle A. Simmons1*  
1 Department of Molecular, Cellular and Developmental Biology  
University of Michigan, 830 North University Ave, Ann Arbor, MI 48109-1048  
2 Department of Biochemistry and Biomedical Sciences, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1 |
| 11:20 a.m. | A SINGLE RESIDUE UNIQUE TO DINB-LIKE PROTEINS LIMITS FORMATION OF THE DNA Pol IV MULTI-PROTEIN COMPLEX IN *ESCHERICHIA COLI*  
Cafarelli, Tiziana M., Thomas J. Rands, Ryan W. Benson, Pamela A. Rudnicki, Ida Lin, and Veronica G. Godoy§  
Department of Biology, Northeastern University, Boston, MA 02115 |
| 11:40 a.m. | TRANSPOSITION TARGETING IS CONTROLLED THROUGH A BALANCE BETWEEN PROTEINS  
Fricker, Ashwana¹, Qiaojuan Shi¹, Adam Parks¹, Joseph Peters¹  
¹Cornell University, Ithaca, NY |
| 12:00 p.m. | LUNCH |
### Keynote Address

Graham C. Walker, Ph.D.
Professor of Biology
Massachusetts Institute of Technology

"Translesion DNA Polymerases, From Cancer Chemotherapy to Antibiotic Action"

---

**Session II – Chair: Michael Fasullo**

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
</table>
| 2:40 p.m. | MODULATION OF MCM2-7 ACTIVITY BY CDT1 | DaSilva, Lance F.¹, Tomasz Kolaczyk¹, Xiaoli Ma¹, Lance D. Langston², Megan J. Davey¹, Michael O’Donnell² and David Edgell¹  
¹Department of Biochemistry, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON N6A 5C1, Canada. ²The Rockefeller University, Howard Hughes Medical Institute, 1230 York Avenue, New York, New York 10065, USA. |
| 3:00 p.m. | DISTINCT MECHANISMS SUPPRESS CHECKPOINT HYPER-SIGNALING DURING DNA REPLICAION | Jablonowski, Carolyn, José Renato Cussiol, Patrice Ohouo, Marcus Smolka. Cornell University |
| 3:20 p.m. | RTT107 RECRUITS SLX4 TO STALLED REPLICAION FORKS VIA γ-H2A | Balint, Attila a,b, Taehyung Kim c,b, Jiongwen Ou a,b, Zhaolei Zhang b,c, Grant W Brown a,b  
a Department of Biochemistry, University of Toronto. b Donnelly Centre for Cellular and Biomolecular Research. c Department of Computer Science, University of Toronto. |
| 3:40 p.m. | GENOME PROFILING OF SACCHAROMYCES CEREVISIAE RESISTANCE TO AFLATOXIN B1 (AFB1), A POTENT LIVER CARCINOGEN THAT TRIGGERS REPLICAION STRESS | Fasullo, Michael a,b, Cinzia Cerab, Jonathan Bard b, Patricia Egner d, Thomas Begley a  
a College of Nanoscale Sciences and b Department of Biomedical Sciences, State University of New York at Albany, c Center of Excellence in Bioinformatics, State University of New York at Buffalo, d Bloomberg School of Public Health, Johns Hopkins University |
| 4:00 p.m. | MUTATIONS IN RAD1 ALTER RAD1-RAD10 ACTIVITY IN 3’ NON-HOMOLOGOUS TAIL REMOVAL | Eichmiller, Robin, Rachel deSanto, Jaime O’Connor, Eugen Minca, Evan Meyers, Mindy Haarmeyer, and Jennifer A. Surtees  
Department of Biochemistry, SUNY Buffalo, Buffalo, NY, USA |

---

**POSTER SESSION, WINE AND CHEESE RECEPTION**

**7:00 p.m.**

**SYMPOSIUM DINNER**

*2ND FLOOR LIBRARY, DOUBLETREE HOTEL*
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Title</th>
<th>Authors</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 a.m.</td>
<td>Continental Breakfast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:20 a.m.</td>
<td>Session III – Chair: Jennifer Surtees</td>
<td>MSH2-MSH3 INTERFERES WITH OKAZAKI FRAGMENT PROCESSING TO PROMOTE TRINUCLEOTIDE REPEAT EXPANSIONS</td>
<td>Williams, Gregory M. and Jennifer A. Surtees</td>
<td>Department of Biochemistry, State University of New York at Buffalo, NY, USA</td>
</tr>
<tr>
<td>8:40 a.m.</td>
<td></td>
<td>ADAPTIVE EVOLUTION IN INCOMPATIBLE DNA MISMATCH REPAIR STRAINS OF BAKER’S YEAST.</td>
<td>Bui, Duyen¹, Elliot Dine¹, James Anderson², Charles Aquadro¹ and Eric Alaní¹</td>
<td>Department of Molecular Biology and Genetics, Cornell University; ²Department of Biology, University of Toronto</td>
</tr>
<tr>
<td>9:00 a.m.</td>
<td></td>
<td>IDENTIFICATION OF A SYNTHETIC LETHAL GENDER BIAS IN A MAMMALIAN MODEL OF DNA REPLICATION</td>
<td>McNairn, Adrian J., Chen-Hua Chuang, Martha Wallace, and John C. Schimenti</td>
<td>Department of Biomedical Sciences, School of Veterinary Medicine, Cornell University, Ithaca, NY</td>
</tr>
<tr>
<td>9:20 a.m.</td>
<td></td>
<td>IDENTIFYING ROLES FOR INTERACTING PARTNERS OF MSH6 AND SGS1 IN HETERO duplex REJECTION IN S. CEREVISIAE</td>
<td>Chakraborty, Ujani, Carolyn M. George, Amy Lyndaker, Eric Alaní</td>
<td>Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY</td>
</tr>
<tr>
<td>9:40 a.m.</td>
<td></td>
<td>ATP AS A LICENSING FACTOR FOR MSH2-MSH3-MEDIATED REPAIR IN VITRO</td>
<td>Kumar, Charanya, Bangchen Wang and Jennifer A Surtees</td>
<td>Department of Biochemistry, State University of New York at Buffalo</td>
</tr>
<tr>
<td>10:00 a.m.</td>
<td>Coffee Break</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:20 a.m.</td>
<td>Session IV – Chair: Anthony Berdis</td>
<td>THE FHA1 DOMAIN FROM Rad53 HAS A UNIQUE INTERACTION SURFACE</td>
<td>Matthews, Lindsay A.¹, Darryl R. Jones², Rajeevan Selvaratnam³, Madoka Akimoto³, Brendan McConkey², Giuseppe Melacini³, Bernard P. Duncker² and Alba Guarné³</td>
<td>¹Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON; ²Department of Biology, University of Waterloo, Waterloo, ON; ³Department of Chemistry, McMaster University, Hamilton, ON</td>
</tr>
<tr>
<td>10:40 a.m.</td>
<td></td>
<td>THE RSC COMPLEX FUNCTIONS TO MAINTAIN PLOIDY IN SACCHAROMYCES CEREVISIAE</td>
<td>Sing, Tina L.¹,², Shinsuke Ohnuki³, Yoshikazu Ohya³ and Grant W. Brown¹,²</td>
<td>¹) Department of Biochemistry, University of Toronto, Canada; ²) Donnelly Centre for Cellular and Biomolecular Research; ³) Department of Integrated Biosciences, University of Tokyo</td>
</tr>
<tr>
<td>11:00 a.m.</td>
<td></td>
<td>HIGH RETROTRANSPOSON COPY NUMBER EXTENDS THE CHRONOLOGICAL LIFESPAN OF SACCHAROMYCES PARADOXUS EXPOSED TO CHRONIC GENOME MAINTENANCE STRESS</td>
<td>Maxwell, Patrick H., David VanHoute and Andrew Peifer</td>
<td>Department of Biology, Rensselaer Polytechnic Institute, Troy, NY</td>
</tr>
<tr>
<td>Time</td>
<td>Title</td>
<td>Authors</td>
<td>Affiliation</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>11:20</td>
<td><strong>FANCM-DEFICIENT MICE REVEAL THE SENSITIVITY OF DNA DAMAGE RESPONSES IN GERMLINE STEM CELLS</strong></td>
<td>Luo, Yunhai, Hartford, Suzanne A., John C. Schimenti</td>
<td>Department of Biomedical Sciences, Cornell University, Ithaca, NY</td>
<td></td>
</tr>
<tr>
<td>11:40</td>
<td><strong>MOLECULAR ANALYSIS OF THE MAMMALIAN DNA DAMAGE CHECKPOINT PROTEIN HUS1</strong></td>
<td>Lim, Pei Xin, Kelsey E Poisson, Manpreet Basuita, Amy M Lyndaker and Robert S Weiss</td>
<td>Department of Biomedical Sciences, Cornell University, Ithaca, New York</td>
<td></td>
</tr>
<tr>
<td>12:00</td>
<td><strong>CHK2 CONTROLS GENOME INTEGRITY OF OOCYTES.</strong></td>
<td>Bolcun-Filas, Ewelina, Vera D. Rinaldi, Michelle White and John C. Schimenti</td>
<td>Department of Biomedical Sciences, Cornell University, Ithaca, NY</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Center for Vertebrate Genomics, Cornell University, Ithaca, NY</td>
<td></td>
</tr>
<tr>
<td>12:20</td>
<td><strong>INCREASED DNA DAMAGE AND OXIDATIVE STRESS IN ALKYLATION REPAIR HOMOLOG 8 (ALKBH8) DEFICIENT MICE</strong></td>
<td>Endres, Lauren, Madhu Dyavaiah, Ulrike Begley, Brian Parr and Thomas Begley</td>
<td>College of Nanoscale Science and Engineering, University at Albany, 257 Fuller Road, Albany NY 12203, USA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Center for Functional Genomics University at Albany, 1 Discovery Drive, Rensselaer, NY 12144, USA</td>
<td></td>
</tr>
<tr>
<td>12:40</td>
<td><strong>Closing Remarks – Bill Burhans</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The beta sliding clamp, encoded by the dnaN gene, is known to interact with several proteins that are required for DNA replication and repair in Escherichia coli. These interactions are conserved across all domains of life. Beta clamp is also implicated in the regulation of polymerase access to the replication fork. The polymerases are considered to be competitive in gaining access to the cleft region of the clamp and the regulation of their concentration in turn regulates this process. In eukaryotes, beta clamp undergoes post translational modifications that regulate the access of polymerases involved in translesion synthesis (TLS). This work aims at understanding the role of beta clamp interactions outside of the cleft that might play a role in regulation of partner access to the DNA. We have identified a group of residues in the beta clamp that are required for the cell survival in the presence of Nitrofurazone (NFZ) and methyl methanesulfonate (MMS). In contrast, these residues are not required for survival after UV treatment. We also demonstrate that a point mutant strain dnaN_E202K that failed to confer the cold sensitivity of umuDC overexpression is sensitive to NFZ and MMS but is not epistatic with dinB (Pol IV). Hda, a homolog of DNA initiator protein DnaA, functions in regulating DnaA-ATP to DnaA-ADP ratio by a process known as Regulatory inactivation of DnaA (RIDA). Our results suggests that the dnaN_E202K strain mimicks overproduction of Hda resulting in reduced number of initiation events. We also show that the E202K mutation results in the modulation of the transcriptional network as a response to replication defect indicating that there is tight coordination between the two processes. This project further aims to characterize the identified mutants genetically and biochemically to understand mechanisms underlying regulation of DNA replication by beta clamp.
PROTEASE STALLING GENERATES CRITICAL CLAMP LOADER DIVERSITY

Vass, Robert H. and Peter Chien
Molecular and Cellular Biology Graduate Program, Department of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA 01003

Chromosome replication relies on sliding clamps that are loaded by energy dependent complexes. In *E. coli*, the ATP-binding clamp loader subunit DnaX exists as both full-length (∥) and short (◎) forms due to a programmed frameshift during translation, but whether both forms are needed is unclear. Here, we show that in *Caulobacter crescentus*, DnaX isoforms are unexpectedly generated through partial proteolysis by the AAA+ protease ClpXP. We take a biochemical approach to show that this normally highly processive protease recognizes a specific region within DnaX, but after limited processing, stalls at a glycine-rich region adjacent to a structured domain. Increasing the sequence complexity of this glycine-rich region results in loss of processing and produces only the ∥-form *in vivo*. We show that strains producing only ∥ fail to grow normally, but providing ◎ in trans restores viability. Interestingly, strains that constitutively express ∥ and ◎ are more sensitive to DNA damaging conditions compared to strains that can dynamically generate ◎. Taken together, our data show that in *C. crescentus*, the clamp loader complex requires proteolytic processing to generate critical subunit diversity. Our results also support a model where different forms of the clamp loader complex are required during response to DNA damage and may represent a generally conserved phenomenon in bacteria.
CONTRIBUTION OF MMR TO DAMAGE INDUCED MUTAGENESIS IN
ESCHERICHIA COLI

Baron, Christopher M. and Mark D. Sutton

Department of Biochemistry, State University of New York at Buffalo

Mismatch repair (MMR) is a repair pathway that acts to ensure genomic fidelity by recognizing and excising errors caused during replication. The result is a single strand DNA gap that is filled by a DNA polymerase, ideally, in an error free manner. Escherichia coli possesses 3 lower fidelity polymerases which are known as Translesion synthesis (TLS) polymerases. These polymerases are regulated by a damage response known as the SOS response, a system which senses DNA damage and upregulates over 40 genes involved in repair of DNA damage. One TLS polymerase, Pol V, is responsible for most mutagenesis catalyzed during the SOS response. I hypothesize that during the SOS response, MMR facilitates SOS mutagenesis by generating a gap that Pol V gains access and catalyzes error prone synthesis. To this end, I have demonstrated that mutations induced by Hydrogen peroxide, Ultra violet radiation and Methyl methanesulfonate requires both MMR protein and activity. Utilizing a construct that constitutively expresses Pol V, I have also demonstrated that mutagenesis catalyzed by Pol V can be further enhanced by stimulation of MMR. I am currently investigating the impact MMR may have on the SOS response, which would influence the level of Pol V present in the cell. My results suggest that MMR facilitates mutagenesis by providing increased opportunity for Pol V to gain access to the DNA template. Future efforts will determine whether loss of MMR affects levels of available Pol V within the cell.
MutS and MutL are highly conserved proteins required for mismatch repair in organisms ranging from bacteria to humans. MutS is responsible for mismatch detection and the recruitment of MutL to mismatch proximal DNA through a mechanism that is unknown in most organisms. Here, we used peptide array mapping and chemical crosslinking experiments to identify the discrete site on MutS that is occupied by MutL. We identified two adjacent phenylalanine residues, F319 and F320, located in an unstructured loop of MutS that facilitate MutL interaction. Disruption of this site renders MutS defective in binding MutL in vitro and broken for recruitment of MutL in vivo, while also eliminating mismatch repair in vivo. Analysis of MutS repair complexes in live cells shows that MutS mutants defective in interaction with MutL are trapped in an unregulated loading cycle in response to mismatch detection. Furthermore, these mutant MutS repair complexes persist on DNA away from the DNA polymerase, suggesting that MutS remains loaded on mismatch proximal DNA awaiting arrival of MutL. Upon arrival of MutL, MutS-DNA bound complexes require a step of repair after MutL recruitment to stimulate disassembly. Furthermore, we present evidence that mismatch excision signals disassembly of MutS complexes in vivo. We also provide evidence showing that MutS and MutL interact independent of mismatch binding by MutS in vivo and in vitro. This finding suggests that MutL transiently probes MutS to determine if MutS is mismatch bound before initiating downstream repair events. Together, these data provide novel insights into the mechanism that MutS employs to recruit MutL, and the consequences that ensue when MutL recruitment fails during mismatch repair in living cells.

§ These authors contributed equally
A SINGLE RESIDUE UNIQUE TO DINB-LIKE PROTEINS LIMITS FORMATION OF THE DNA Pol IV MULTI-PROTEIN COMPLEX IN ESCHERICHIA COLI

Cafarelli, Tiziana M., Thomas J. Rands, Ryan W. Benson, Pamela A. Rudnicki, Ida Lin, and Veronica G. Godoy #
Department of Biology, Northeastern University, Boston, MA 02115

The activity of DinB is governed by the formation of a multi-protein complex (MPC) with RecA, the cell’s main recombinase, and UmuD, an accessory subunit. We identified two highly conserved surface residues of DinB, Cysteine 66 (C66) and Proline 67 (P67). Mapping on the DinB tertiary structure suggests these residues are non-catalytic. Moreover, multiple sequence alignments indicate that they are unique among DinB-like proteins. We examined their role in the formation of regulatory complexes, focusing on C66. We constructed the dinB(C66A) allele to investigate the role of the C66-containing surface in DinB MPC formation. We find that DinB(C66A) co-purifies with its interacting partners, RecA and UmuD, to a greater extent than DinB. In vitro pulldown assays also indicate that DinB(C66A) binds RecA and UmuD better than DinB. We note that the increased affinity of DinB(C66A) for UmuD is RecA-dependent. Thus, the C66-containing binding surface appears to be critical to modulate interaction with UmuD and particularly with RecA. Expression of dinB(C66A) from the chromosome resulted in detectable differences in dinB-dependent lesion bypass fidelity and homologous recombination. We also determined the kinetic parameters of dNTP insertion opposite adenine or 3-deaza-3-methyladenine, an alkylation lesion analogue, in vitro. Interestingly, we find that both the native and mutant proteins proficiently replicate both undamaged and lesion-containing template with high fidelity, but that DinB(C66A) is less efficient than DinB. Study of this DinB derivative has revealed a key surface on DinB, which appears to modulate the strength of MPC binding, and has suggested a binding order of RecA and UmuD to DinB. These findings will ultimately permit the manipulation of these enzymes to deter bacterial antibiotic resistance acquisition and to gain insights into cancer development in humans.
TRANPOSITION TARGETING IS CONTROLLED THROUGH A BALANCE BETWEEN PROTEINS
Fricker, Ashwana 1, Qiaojuan Shi1, Adam Parks1, Joseph Peters1
1Cornell University, Ithaca, NY

Transposons are DNA segments that can move between sites within a cell. Tn7 and Tn7-like elements are bacterial transposons that have the capacity to target plasmids capable of moving between bacteria called mobile plasmids by identifying aspects of DNA replication. These elements are broadly distributed among diverse bacteria in disparate environments and play an important role in bacterial adaptation (acquisition of pathogenesis, biosynthesis pathways, host defenses, among many more). Work with Tn7 indicates tight control over the frequency and localization of transposition events as orchestrated by five transposon-encoded proteins. Two of these transposon encoded proteins, TnsC (regulation) and TnsE (targeting), function together to direct transposition into actively replicating mobile plasmids.

We show here that the functional interaction between TnsE and TnsC is predicated on a sensitive balance between the DNA binding abilities of the proteins. By using gain-of-activity mutations in both proteins, we are able to dial between random and highly targeted transposition. TnsE has a specific preference for DNA structures with 3'-recessed ends, associated with lagging strand DNA replication. Our work indicates that the stability of TnsE binding to its preferred DNA substrate is a critical component for controlling both the frequency and targeting of Tn7 transposition. TnsE mutants isolated for the ability to allow high levels of transposition and strong targeting to DNA replication show drastically increased binding stability to the preferred DNA target. Of further interest, alleles found in our genetic screen also appear to occur naturally in the environment. One allele in particular which is critical in the balance between TnsC and TnsE may contribute to the wide distribution of the Tn7 family of elements. A model is proposed for how the interplay between TnsC and TnsE and the DNA binding abilities allow for the evolution of targeting pathways to suit the selection environment.

"This work was funded in part by the NSF MCB1244227"
MODULATION OF MCM2-7 ACTIVITY BY CDT1

DaSilva, Lance F. 1, Tomasz Kolaczyk1, Xiaoli Ma1, Lance D. Langston2, Megan J. Davey1, Michael O’Donnell2 and David Edgell1
1Department of Biochemistry, Schulich School of Medicine and Dentistry, University of Western Ontario, London, ON N6A 5C1, Canada. 2The Rockefeller University, Howard Hughes Medical Institute, 1230 York Avenue, New York, New York 10065, USA.

Genome duplication occurs once and only once in each cell cycle. During G1 phase, pre-replicative complexes (pre-RC), composed of ORC, Cdc6, Cdt1 and Mcm2-7, are assembled at replication origins. Mcm2-7 is the replicative helicase in eukaryotic cells; however, it does not unwind DNA in the pre-RC. It is only in S-phase, after activation of Mcm2-7, that DNA unwinding is detected. Studies show that Cdt1 plays an important role in stabilizing Mcm2-7 on ORC•Cdc6•DNA. Moreover, Cdc6 and Orc1 ATPase activity are equally important to facilitate Cdt1 release and Mcm2-7 origin loading. Using purified components from bacterial expression systems, we assembled Mcm2-7•Cdt1 complexes and compared its activity to that of Mcm2-7 alone. We show that Mcm2-7•Cdt1 has lower ATPase activity than Mcm2-7. In addition, DNA unwinding by Mcm2-7•Cdt1 is significantly lower than by Mcm2-7 alone. Furthermore, in vitro pre-RC reconstitution experiments showed that loading of Mcm2-7 onto origins was dependent on ORC, Cdc6 and Cdt1. Our data suggest that the regulatory role of Cdt1 in the initiation of DNA replication is mediated largely through an inhibition of Mcm2-7 activity.
DISTINCT MECHANISMS SUPPRESS CHECKPOINT HYPER-SIGNALING DURING DNA REPLICATION

Jablonowski, Carolyn, José Renato Cussiol, Patrice Ohouo, Marcus Smolka. Cornell University

Genomic integrity is especially vulnerable during DNA replication. In response to replication stress, DNA damage checkpoint (DDC) kinases are activated and coordinate a wide range of cellular responses necessary for genome maintenance and cell survival. In *S. cerevisiae*, the key DDC kinase Rad53 plays crucial roles in the regulation of transcription, dNTP levels, cell cycle progression and origin firing. Hyperactivation of Rad53 has been shown to be deleterious and result in sensitivity to replication stress, highlighting the importance of mechanisms for regulating Rad53 activation. Our lab recently revealed a phosphatase-independent mechanism, mediated by the DNA repair scaffold Slx4, which suppresses aberrant hyperactivation of DDC signaling (Ohouo et al 2012). In this mechanism, Slx4 competes with the checkpoint adaptor Rad9 for the binding to the mediator protein Dpb11, and therefore blocks signal transduction from the upstream kinase Mec1 to Rad53. Here we show that cells expressing an allele of *SLX4* (*slx4*-S486A) that cannot bind Dpb11 phenocopy cells lacking *PPH3*, the main phosphatase that acts on Rad53. Both cells are sensitive to the DNA alkylation drug methyl methanesulfonate (MMS) but not to other replication stress-inducing agents such as hydroxyurea or camptothecin. Following MMS treatment, *slx4*-S486A and *pph3Δ* cells show hyperactivation of Rad53 and an intra-S phase cell cycle delay. Strikingly, mutation of two phosphorylation sites in Rad9 that prevents its interaction with Dpb11 rescues these phenotypes, showing that these problems may arise due to hyperactivation of Rad53 via the Dpb11-Rad9 pathway. Together, these results show that there are multiple mechanisms that act to control the activation of Rad53 at replication-bypassed lesions, and that hyperactivated Rad53 leads to problems during intra-S-phase that are independent of a persistent G2/M checkpoint arrest. The mechanism of how Rad53 activity leads to these intra-S defects is not yet understood.
RTT107 RECRUITS SLX4 TO STALLED REPLICATION FORKS VIA γ-H2A

Balint, Attila a,b, Taehyung Kim b,c, Jiongwen Ou a,b, Zhaolei Zhang b,c, Grant W Brown a,b

a Department of Biochemistry, University of Toronto. b Donnelly Centre for Cellular and Biomolecular Research. c Department of Computer Science, University of Toronto.

DNA replication errors are a major source of genome instability, a hallmark of cancer and other human genetic diseases. Many cellular and environmental agents generate DNA lesions that can cause replication forks to stall. Replication fork stalling is sensed by the DNA replication checkpoint, an evolutionarily conserved signaling pathway that acts to facilitate the completion of DNA replication. In S. cerevisiae, two important targets of this checkpoint are the Rtt107 and Slx4 scaffolds, binding partners that are thought to function together during replication stress by coordinating DNA damage response enzymes. Previously, our lab showed that Rtt107 localizes to origin-proximal regions during DNA replication stress. However, whether Slx4 shows a similar localization and how Rtt107 (and Slx4) recruitment to these sites is regulated has yet to be clearly defined.

Utilizing chromatin immunoprecipitation coupled to deep sequencing (ChIP-seq), we have found that Slx4, like Rtt107, localizes to regions of replication fork stalling by the DNA alkylating agent methyl methanesulfonate (MMS). Recruitment of Slx4 to stalled forks requires a physical interaction with Rtt107, whereas Rtt107 localization is largely unaffected in slx4Δ cells, suggesting that Rtt107 is upstream of Slx4 in the recruitment pathway. Consistent with recent in vitro binding studies, Rtt107 recruitment is dependent on histone H2A phosphorylated on Ser129 (γH2A), an early phosphorylation event during the replication checkpoint response. Furthermore, MMS-dependent Rtt107 and Slx4 phosphorylation is largely eliminated in h2a-s129a cells, suggesting that phosphorylation of these scaffolds occurs only when they are bound near stalled forks. Interestingly, DNA replication stress-induced binding of Slx4 to Dpb11, a replication checkpoint activation factor, is dispensable for recruitment of Slx4 to stalled forks. Nonetheless, physical interactions with both Rtt107 and Dpb11 are important for Slx4 function in resistance to MMS. Together, our data establish the molecular mechanism of Rtt107-Slx4 recruitment to stalled replication forks and illustrate the importance of the Rtt107-Slx4 physical interaction during DNA replication stress.
GENOME PROFILING OF SACCHAROMYCES CEREVISIAE RESISTANCE TO AFLATOXIN B1 (AFB1), A POTENT LIVER CARCINOGEN THAT TRIGGERS REPLICATION STRESS.

Fasullo, Michael\textsuperscript{a,b}, Cinzia Cera\textsuperscript{b}, Jonathan Bard\textsuperscript{c}, Patricia Egner\textsuperscript{d}, Thomas Begley.\textsuperscript{a}\textsuperscript{a}College of Nanoscale Sciences and \textsuperscript{b}Department of Biomedical Sciences, State University of New York at Albany, \textsuperscript{c}Center of Excellence in Bioinformatics, State University of New York at Buffalo, \textsuperscript{d}Bloomberg School of Public Health, Johns Hopkins University

The mycotoxin aflatoxin B1 (AFB1) is the most potent liver carcinogen. AFB1 is a contaminant in rotting grain and poses an increasing risk due to global warming. A signature p53 mutation is found in tumors from liver cancer patients who inhabit AFB1-contaminated areas, suggesting that AFB1 is a potent genotoxin. P450 enzymes convert AFB1 into a highly reactive epoxide that forms N\textsuperscript{7}-guanine DNA adducts, which are unstable and convert into AFB1-formamidopyrimidine (FAPY)-derivatives. These AFB1-associated adducts are either mutagenic or block DNA replication \textit{in vitro}. In CYP1A2-expressing budding yeast, AFB1 is a weak mutagen but a potent recombinagen, and triggers the formation of recombinational repair foci. A DNA damage-checkpoint response correlates with a delay in cell cycle progression. Microarray analysis reveals that both DNA repair and stress response genes are up regulated. To elucidate the functional significance of transcriptional induction, we are profiling the yeast genome for AFB1 resistance, using state-of-the-art next generation sequencing to identify molecular barcodes. We introduced the human CYP1A2 into \textasciitilde90\% of the deletion library, and pooled samples have been exposed to 50 \textmu M and 100 \textmu M AFB1 for 20 hrs. We identified genes that confer resistance to AFB1 by barcode sequencing, and additional genes by growth curves. DNA metabolism genes include \textit{RAD55, RAD17, REV1,} and \textit{REV3,} emphasizing the potency of the mycotoxin to trigger replication stress. Additional genes include those that function to elevate deoxynucleotides, maintain mitochondria, promote cellular growth and rearrange the cytoskeletal architecture. Current efforts are focused on identifying additional resistance genes by exposing cells to AFB1 for fewer generations and using systems approaches for defining functional relationships. The ultimate aim will be to identify corresponding mammalian genes. The yeast libraries will be valuable for additional high-throughput studies using other metabolically-activated drugs and carcinogens. Grant support: National Institutes of Health: R21ES1954, F33ES021133
MUTATIONS IN RAD1 ALTER RAD1-RAD10 ACTIVITY IN 3’ NON-HOMOLOGOUS TAIL REMOVAL

Eichmiller, Robin, Rachel deSanto, Jaime O’Connor, Eugen Minca, Evan Meyers, Mindy Haarmeyer, and Jennifer A. Surtees

SUNY Buffalo, Buffalo, NY, USA

Saccharomyces cerevisiae Rad1-Rad10 is a structure specific endonuclease that cleaves at double strand/single strand junctions with 3’ single-stranded DNA. Rad1-Rad10 is essential for at least three distinct DNA repair pathways: nucleotide excision repair (NER), interstrand crosslink repair (ICLR), and a specialized form of double strand break repair (DSBR) by homologous recombination that involves 3’ non-homologous tail removal. Rad1-Rad10 interacts with and is modulated by different proteins in each pathway. Importantly, Rad1-Rad10 requires protein partners for its recruitment to DNA lesions. Rad14 is required for Rad1-Rad10 recruitment in NER; Saw1 and/or Msh2-Msh3 do the same in DSBR; currently, no recruitment partner has been identified in ICLR.

Understanding the coordination and regulation of Rad1-Rad10 in these pathways is critical. By targeting the putative DNA-binding region of RAD1, we have identified separation-of-function mutations through in vivo analysis. rad1R203A K205A is functional for ICLR, has an intermediate phenotype for NER, and is non-functional in DSBR. rad1R218A is functional for ICLR and NER, but not DSBR. The phenotypes indicate that both mutant complexes retain some enzymatic activity. Therefore we hypothesize that the regulation and/or recruitment of Rad1-Rad10 to distinct DNA substrates has been affected. To test this, we have purified the mutant complexes and characterized their in vitro endonuclease and DNA-binding activities. Both mutants retain DNA binding activity and rad1R218A-Rad10 retains wild type levels of endonuclease activity. rad1R203A K205A-Rad10 has a 2-3 fold reduction in endonuclease activity on both the NER and DSBR DNA substrates. We are currently testing the protein-protein interactions of the mutants. We hypothesize that rad1R218A is defective for 3’NHTR is due to altered protein-protein interactions and that changes in the confirmation of rad1R203A K205A alters its interaction with its DNA substrates leading to a defect. 3’NHTR may be more sensitive to this defect than NER. (Work in the Surtees lab is supported by NIH-GM-87459)
Trinucleotide repeat (TNR) expansions are the underlying cause of more than forty neurodegenerative and neuromuscular diseases, including myotonic dystrophy and Huntington’s disease. Although genetic evidence has implicated errors in DNA replication and/or DNA repair as an important cause of TNR expansions, no clear molecular mechanism has been elucidated. We have shown previously that sequence expansions can occur when the mismatch repair complex Msh2-Msh3 interferes with proteins involved in Okazaki fragment processing in *Saccharomyces cerevisiae*. In vivo, we found that Msh2-Msh3 is involved in TNR expansions in *S. cerevisiae*, in regions containing CTG or CAG repeats on the DNA lagging strand. Our *in vitro* results indicated that Msh2-Msh3 interacts with replication intermediates containing TNRs and modulates the activity of flap endonuclease1 and DNA Ligase I. During *in vitro* Okazaki fragment processing of replication intermediates containing TNRs, Msh2-Msh3 promoted incremental expansions. We have performed a series of time course experiments to document the TNR tract dynamics and to determine whether incremental expansions also occur *in vivo*. We have followed the size of TNR tracts over time in the presence and absence of Msh3 and have observed dynamic changes within the tract. Here we show that TNR sequences in a wild type background readily accumulate small incremental expansions over time in a series of small expansion and contraction events. Very few expansions accumulated in a *msh3Δ* background. These experiments display the dynamics of TNR expansions and contractions, and help elucidate the role of Msh2-Msh3 in shifting the expansion-contraction equilibrium towards expansion (NIH-GM-87459).
ADAPTIVE EVOLUTION IN INCOMPATIBLE DNA MISMATCH REPAIR STRAINS OF BAKER’S YEAST.

Bui, Duyen¹, Elliot Dine¹, James Anderson², Charles Aquadro¹ and Eric Alani¹

¹Department of Molecular Biology and Genetics, Cornell University; ²Department of Biology, University of Toronto

DNA mismatches can arise as the result of DNA replication errors. Mismatch repair (MMR) proteins serve to repair such errors to ensure high replication fidelity. Previously we identified negative epistatic interactions between the MLH1 and PMS1 MMR genes of baker’s yeast. Specifically, we found that the Mlh1-Pms1 complex can exhibit defects in MMR functions when its subunits are assembled from different yeast strains. We refer to the strains bearing such heterodimers as “incompatible strains”. Such strains display high mutation rates and show a long term fitness cost. The Alani lab has proposed a model in which two geographically isolated populations arising from a common ancestor can create a hybrid mutator through mating. Furthermore, we hypothesize that a MMR incompatibility in budding yeast can initially allow more rapid adaptation to a new environment. However, once adapted to an environment, the accumulation of deleterious mutations could eventually outweigh fitness advantages of the incompatible population. Consistent with this model, my data show that incompatible strains can adapt more rapidly to a stress environment (high salt), suggesting that MMR incompatibilities can play a significant role in adaptive evolution. Furthermore, the data suggest that this advantage is rapid but transient. At present we are looking for the causative mutations of the observed fitness advantage in stress environments.
IDENTIFICATION OF A SYNTHETIC LETHAL GENDER BIAS IN A MAMMALIAN MODEL OF DNA REPLICATION

McNairn, Adrian J., Chen-Hua Chuang, Martha Wallace, and John C. Schimenti
Department of Biomedical Sciences, School of Veterinary Medicine, Cornell University, Ithaca, NY

The minichromosome maintenance proteins (MCM) form a highly conserved heterohexameric complex that is essential for DNA replication. We have previously characterized a hypomorphic MCM allele, MCM4\textsubscript{Chaos3} (hereafter referred to as Chaos3), that results in reduced mRNA and protein levels of other MCM subunits. Additionally, >80% of female Chaos3 mice develop mammary tumors at 1 year of age. To identify synthetic lethal interactions among mammalian MCM subunits, Chaos3 mice were crossed to mice containing genetraps of other MCM genes. Through this complex haploinsufficiency in the MCM genes, a female specific synthetic lethality was observed. This effect of MCM haploinsufficiency could be alleviated using an SRY transgene or by treating pregnant females with testosterone. To further characterize the mechanism of this sex bias, MEFs containing the homozygous Chaos3 MCM4 allele, in combination with the loss of an MCM2 allele, were treated with testosterone \textit{in vitro}. These cells exhibited an increase in the protein levels of androgen receptor, but not MCMs, without an increase in mRNA levels, indicating the effect of testosterone is mediated via translation. Examination of cell signaling pathways revealed a down-regulation of the TGF\textbeta pathway in MCM-deficient female MEFs treated with testosterone. These results indicate the presence of a sex-specific feedback loop between MCM proteins levels and the TGF\textbeta pathway that may regulate tissue proliferation and development.
IDENTIFYING ROLES FOR INTERACTING PARTNERS OF MSH6 AND SGS1 IN HETERODUPLEX REJECTION IN S. CEREVISIAE

Chakraborty, Ujani, Carolyn M. George, Amy Lyndaker, Eric Alani

Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY

In baker’s yeast the mismatch recognition factor Msh2-Msh6 and the Sgs1 helicase suppress genetic recombination between divergent repetitive DNA sequences. Such DNA sequences pose risks to eukaryotic cells because they have the potential to recombine and yield deleterious chromosomal rearrangements. We analyzed the roles of Msh2-Msh6 and Sgs1 in preventing recombination between divergent sequences using a single strand annealing (SSA) repair assay. In this system Msh2-Msh6 and Sgs1 have been proposed to prevent recombination between divergent DNA sequences by unwinding heteroduplex DNA intermediates containing DNA mismatches (Sugawara et al. PNAS 101:9315, Goldfarb et al. Genetics 169:563). To further test this model, we are currently testing protein interactors of Msh6 and Sgs1. Here we show that the Top3-Rmi1 topoisomerase, which interacts with Sgs1, is critical to prevent recombination between divergent sequences. We also tested the role of the DNA replication processivity clamp PCNA in this system because it forms a complex with Msh2-Msh6 and was shown to be important for facilitating mismatch recognition in vitro (Flores-Rozas et al. Nat Genet 26:375). We found that the interaction between Msh2-Msh6 and PCNA appeared dispensable for preventing recombination between divergent sequences. However, PCNA was found to be important for repairing base pair mismatches created by SSA. Moreover, some pol30 alleles conferred a general defect in SSA. Together our work suggests that Msh2-Msh6 and Sgs1-Top3-Rmi1 are part of a minimal machinery required for the unwinding of heteroduplex DNA containing mismatches, and that they likely participate in this process through direct physical interactions. Our study also supports previous work showing that PCNA is not required for regulating the fidelity of homologous recombination, but is required for correcting mismatches generated during homologous recombination, most likely in steps following mismatch recognition (Stone et al. Genetics 178:1221, Lau et al. MCB 22:6669).
ATP AS A LICENSING FACTOR FOR MSH2-MSH3-MEDIATED REPAIR IN VITRO

Kumar, Charanya, Bangchen Wang and Jennifer A Surtees
Department of Biochemistry, State University of New York at Buffalo

Mismatch repair (MMR) is an evolutionarily conserved DNA repair pathway that directs repair of errors that occur during replication. It improves the fidelity of replication by two to three orders of magnitude and is critical for maintaining genome stability. In *Saccharomyces cerevisiae*, MMR is initiated by one of two heterodimeric complexes: Msh2-Msh6 or Msh2-Msh3. Repair is initiated once these complexes recognize and bind the mispair. Msh2-Msh6 binds and directs repair of base-base mismatches and small (1-2 nt) insertion/deletion loops (IDL) while Msh2-Msh3 initiates repair of IDLs up to 17 nucleotides long. In addition to MMR, Msh2-Msh3 is involved in 3’ non-homologous tail removal (3’NHTR), a step in specialized forms of double-strand break repair pathways that generate intermediates with 3’ non-homologous tails, such as single-strand annealing. Msh2-Msh3 also prevents recombination between similar but non-identical sequences by a pathway called heteroduplex rejection. Improper function of Msh2-Msh3 can thus lead to genomic instability.

*In vivo* and *in vitro* evidence has indicated that two key functions of Msh2-Msh3, DNA binding and ATP hydrolysis are required for proper function in these pathways and these two functions modulate each other. We recently showed that *in vivo* the requirements for the nucleotide-binding pocket of Msh3 are different for 3’NHTR, heteroduplex rejection and MMR. We hypothesized that the differential requirements for nucleotide binding might result from differential regulation of ATP binding and/or hydrolysis by the different DNA substrates associated with each pathway. We have used a coupled spectroscopic method to measure the ATPase activity of yeast Msh2-Msh3 under a variety of different conditions. We have found that the kinetic parameters that govern the ATPase activity of the enzyme are distinct between the DNA substrates. Furthermore, we have found that the affinity of Msh2-Msh3 for nucleotide depends on the DNA substrate present. Our data support a model in which Msh2-Msh3 uses ATP binding and/or hydrolysis as a licensing factor for repair. (NIH GM 87459)
THE FHA1 DOMAIN FROM Rad53 HAS A UNIQUE INTERACTION SURFACE

Matthews, Lindsay A.1, Darryl R. Jones2, Rajeevan Selvaratnam3, Madoka Akimoto3, Brendan McConkey2, Giuseppe Melacini3, Bernard P. Duncker2 and Alba Guarné1.
1) Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON. 2) Department of Biology, University of Waterloo, Waterloo, ON. 3) Department of Chemistry, McMaster University, Hamilton, ON.

ForkHead Associated (FHA) domains mediate protein-protein interactions and are found in all kingdoms of life. In eukaryotic cells, FHA domains are enriched in proteins that respond to DNA damage. Their traditional role is recognizing phospho-epitopes that can be created by stress-activated kinases. However, individual FHA domains often interact with multiple partners and how this is orchestrated in the cell is poorly understood. We have investigated a critical interaction during replication stress in budding yeast between the FHA1 domain of the kinase Rad53 and its substrate Dbf4. This prevents late origin firing and allows the cell time to recover from stress before resuming replication (1-4). We have previously reported that this interaction depends on a Dbf4 domain that does not harbor a phospho-epitope, suggesting it may reveal a new mode of FHA domain binding (5). Through the use of NMR spectroscopy, chemical cross-linking, bioinformatics, and yeast two-hybrid analyses, we have mapped the interaction interface on the Rad53 FHA1 domain. Rad53 interacts with Dbf4 through a non-canonical interface that does not involve the phospho-epitope binding site. Indeed, Rad53 can interact with Dbf4 and a phosphopeptide simultaneously, confirming that they bind different surfaces of the FHA1 domain. Furthermore, FHA1 mutations that destroy phospho-epitope recognition do not hinder the interaction with Dbf4 in vitro. However, phospho-epitope recognition is still required in vivo (6). We therefore propose that the FHA1 domain from Rad53 acts as an AND logic gate in the replication stress checkpoint. The interaction with Dbf4 depends on both the phospho-epitope binding site and the non-canonical surface that we have identified. This reveals the intricacy of how FHA domain interactions are regulated in the cell, which extends beyond phospho-epitope recognition.

Ploidy is tightly regulated in eukaryotic cells, and is critical for cell function and survival. Cells must coordinate multiple pathways to ensure replicated DNA is segregated both accurately and in a timely fashion to prevent changes in chromosome number. Several cellular processes have been implicated in ploidy maintenance, including spindle pole body (SPB) duplication, mitotic spindle formation and kinetochore attachment.

In this study, we discovered an unanticipated role for 6 non-essential subunits of the RSC (Remodels the Structure of Chromatin) complex in ploidy maintenance. Using flow cytometry, we demonstrated that deletion of \textit{RSC1}, \textit{RSC2}, \textit{RSC30}, \textit{LDB7}, \textit{NPL6}, or \textit{HTL1}, causes a rapid transition from haploid to diploid DNA content following germination of haploid mutant spores. We used comparative genome hybridization on tiling microarrays to show that the resulting diploids contained the full complement of genes in two copies, were not aneuploid at any loci, and so are true diploids. However, unlike normal diploids that arise from mating, RSC mutant diploids retained the ability to mate with haploids of the opposite mating type, indicating that diploidization is the result of endo-reduplication or complete mis-segregation of the DNA at mitosis, rather than a mating-type switching event.

Morphological analysis of RSC mutants indicates key differences between RSC mutants and normal diploids. We are using morphological data in combination with other functional genomics analyses to identify the complete complement of genes that influence ploidy maintenance in budding yeast.

Interestingly, we find that RSC mutants that diploidize have defects in SPB duplication and separation, yet have not identified effects on SPB gene expression. This suggests that the RSC complex could have roles in chromosome segregation that are independent of RSC function in transcription and at the kinetochore. Future experiments will be directed at gaining mechanistic insight into how the RSC complex facilitates proper SPB duplication and function.

We anticipate that identifying the key pathways that regulate ploidy will yield an integrated view of how these pathways converge to maintain the haploid state and propagate it from one generation to the next.
HIGH RETROTRANSPOSON COPY NUMBER EXTENDS THE
CHRONOLOGICAL LIFESPAN OF SACCHAROMYCES PARADOXUS
EXPOSED TO CHRONIC GENOME MAINTENANCE STRESS

Maxwell, Patrick H., David VanHoute, Andrew Peifer
Department of Biology, Rensselaer Polytechnic Institute, Troy, NY

Replication of retrotransposons through an RNA intermediate has had a substantial influence on genome organization and genetic diversity in eukaryotic organisms. The potential for the presence of retrotransposons in genomes and the genetic damage that they can cause to influence cellular and organismal aging has not been well addressed. Most model organisms have many genomic copies of retrotransposons, but we are making use of a previously described Saccharomyces paradoxus (baker’s yeast) strain that lacks active retrotransposons. We have generated derivatives of this strain that harbor one or more chromosomal copies of a Saccharomyces cerevisiae Ty1 retrotransposon to test the influence of Ty1 copy number and retrotransposition on yeast aging. Yeast chronological aging occurs as cells progressively lose viability in nutrient-depleted saturated cultures. We have found that S. paradoxus strains with zero, low (1-3), or high (>20) Ty1 copy number have similar chronological lifespans when grown in standard rich medium. When strains are grown in medium containing a low concentration of hydroxyurea (an inhibitor of dNTP synthesis), high Ty1 copy number strains have a longer chronological lifespan than low or zero Ty1 copy number strains. Lifespan was not reduced in strains with low Ty1 copy number, and this was unexpected, since a copy-number control mechanism results in high levels of retrotransposition in strains with few Ty1 elements and low levels of retrotransposition in strains with many Ty1 elements. We have confirmed that Ty1 mobility is much higher in strains with low Ty1 copy number compared to strains with high Ty1 copy number, and mobility is further increased by hydroxyurea treatment. We are currently testing whether low Ty1 copy number strains have higher levels of genome instability. We are also further exploring the longer lifespan of high Ty1 copy number strains when treated with hydroxyurea. Both increases in reactive oxygen species (ROS) levels and failure of cells to arrest during G1 phase of the cell cycle have been associated with accelerated chronological aging. Ongoing experiments are investigating whether the longer lifespans of the high Ty1 copy number strains are due to changes in ROS levels or more frequent arrest in G1 during stationary phase.
**FANCM-DEFICIENT MICE REVEAL THE SENSITIVITY OF DNA DAMAGE RESPONSES IN GERMLINE STEM CELLS**

Luo, Yunhai, Hartford, Suzanne A., John C. Schimenti
Department of Biomedical Sciences, Cornell University, Ithaca, NY

The germline is the group of cells which carries and passes genetic information from one generation to the next. Because of this important role, it is critical for germ cells to maintain their genomes well. Indeed, there is evidence showing that spontaneous mutation rates in germ cells are lower than those in somatic cells. However, the mechanism behind such protection is not fully understood. Here, we characterized a mutant mouse *Fancm*\textsuperscript{Chaos4/Chaos4}, which might affect genome maintenance in primordial germ cells. *Fancm* is a member of the Fanconi Anemia complementation group, which is responding to stalled replication forks during DNA replication. Gonadal abnormalities and reduced fertility, which are common in FA patients, have been observed in all FA mouse models. In agreement with this, a point mutation in *Fancm, Chaos4*, causes gonadal abnormality. Immunohistochemistry analysis of newborn gonads revealed a germ cell depletion, suggesting the defect may originate in the primordial germ cell population during embryogenesis. We narrow down the critical period for this phenotype to between E11.5 and E12.5 days during embryogenesis. Primary studies suggest the germ cell depletion might be related to reduced cell proliferation, but not germ cell apoptosis. We hypothesize that the *Fancm*\textsuperscript{Chaos4} mutation may directly correlate to elevated DNA damage at the cellular level and that sensitive DNA damage responses in germ cells lead to a germ cell depletion phenotype. To test this hypothesis, we reasoned that genetic ablation of checkpoint signaling would rescue the germ cell depletion phenotype. Indeed, *Fancm* and *p53* compound mutants have a normal germ cell number in newborn gonads. *p21* mutation partially rescued the germ cell depletion of *Fancm*\textsuperscript{Chaos4/Chaos4} mice. These data suggests that *p53*-p21 signaling might trigger germ cell depletion to protect germline genome in response to the deleterious consequences of *Fancm*\textsuperscript{Chaos4} mutation.
MOLECULAR ANALYSIS OF THE MAMMALIAN DNA DAMAGE CHECKPOINT PROTEIN HUS1
Lim, Pei Xin, Kelsey E Poisson, Manpreet Basuita, Amy M Lyndaker and Robert S Weiss
Department of Biomedical Sciences, Cornell University, Ithaca, New York.

The RAD9-HUS1-RAD1 (9-1-1) complex is a heterotrimeric DNA clamp that promotes DNA damage checkpoint signaling and repair. Loading of the 9-1-1 complex onto damaged DNA is required for cell survival following genotoxic stress. It is well established that the 9-1-1 complex stimulates ATR checkpoint signaling through interactions with the ATR activator TOPBP1. However, its functions in DNA repair are less well understood. The 9-1-1 complex shares structural homology with proliferating nuclear cell antigen (PCNA) and, like PCNA, it interacts with many DNA repair proteins. However the physiological significance of these interactions remains untested in most cases. We hypothesize that, like PCNA, the 9-1-1 complex acts as a molecular scaffold for the recruitment of repair complexes to DNA damage sites. To elucidate HUS1 molecular mechanisms, we tested the ability of various targeted HUS1 mutants to complement the genotoxin sensitivity of Hus1-deficient fibroblasts. Multiple positively-charged HUS1 residues positioned on the inner surface of 9-1-1 ring were found to be functionally important and matched those predicted by computational modeling of the 911-FEN1-DNA ternary structure. These HUS1 residues functioned cooperatively, likely contributing to DNA binding in a synergistic manner. Interestingly, we also discovered functionally important charged residues not predicted by the computational model, and additionally observed several mutants that rendered cells hypersensitive to replication stress but not to bulky DNA lesions, suggesting that different types of DNA damage may necessitate distinct conformations or amounts of HUS1 on chromatin. On its exterior surface, HUS1 contains a hydrophobic pocket that is topologically analogous to the PCNA interacting protein (PIP)-binding pocket of PCNA, but mutagenesis of this region suggested that it is not critical for HUS1 function. Consistent with this finding, multiple sequence alignments revealed that this region is evolutionarily conserved in PCNA and RAD9, but not HUS1. These findings suggest that HUS1 might use a different mechanism to associate with binding partners. Indeed we identified another hydrophobic pocket on HUS1 that is evolutionarily conserved and are currently testing its functional significance. In conclusion, HUS1 potentially engages DNA in different conformations, which we speculate may influence how 9-1-1 interacts with particular effectors. Furthermore, the sites for protein-protein associations on the HUS1 outer surface appear to be distinct from those of PCNA and RAD9, prompting further studies into the molecular mechanisms of HUS1-mediated DNA damage responses.
Oocytes and sperm each contribute half of the genome to the new organism. However, majority of genetic abnormalities can be traced to the maternal side suggesting weaker quality checkpoints in the oocyte. Germ cells undergo two critical processes during meiosis: chromosome synapsis and homologous recombination. Completion of both depends on the generation of Double Strand Breaks (DSB) - DNA damage that has to be repaired to form crossovers. Oocytes failing to do so are eliminated by the DNA damage checkpoint. Occasionally, damaged oocytes evade checkpoint and can carry genetic abnormalities to the next generation. In severe situations checkpoint eliminates all oocytes leading to infertility. Here, we will present the mechanism of the DNA damage checkpoint in the oocyte and its implications.
INCREASED DNA DAMAGE AND OXIDATIVE STRESS IN ALKYLATION REPAIR HOMOLOG 8 (ALKBH8) DEFICIENT MICE

Endres, Lauren¹*, Madhu Dyavaiah¹, Ulrike Begley¹, Brian Parr² and Thomas Begley¹

¹College of Nanoscale Science and Engineering, University at Albany, 257 Fuller Road, Albany NY 12203, USA, ²Center for Functional Genomics University at Albany, 1 Discovery Drive, Rensselaer, NY 12144, USA

*Corresponding author. Tel.: (518) 437-8839; fax: (518) 437-8687; e-mail address: lendres@albany.edu

Alkbh8 belongs to the ABH family of enzymes (Abh1 to 8) found in mice and humans and was computationally identified as a 2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase. Some members of the ABH family function in direct DNA alkylation base repair through the oxidative demethylation of 1-methyladenine (1-meA) and 3-methylcytosine (3-meC) in DNA and RNA. However, Alkbh8 is unique among the family because it contains an RNA binding motif and methyltransferase domain. Both Alkbh8 and its homolog in yeast, Trm9, catalyze the formation of 5-methylcarboxymethyl uridine (mcm5U) and 5-methylcarbonylmethyl-2-thiouridine (mcm5s2U) at the wobble position of tRNAs encoding arginine, glutamic acid, glycine and selenocysteine. We have previously demonstrated that Trm9 optimizes the DNA damage response by promoting the translation of key stress response transcripts such as RNR1 and RNR3. Here we have investigated whether Alkbh8 functions in the mammalian DDR using a mouse gene trap deletion (knockout) mouse model and show that cells from Alkbh8⁻/⁻ mice have cellular and molecular signatures indicative of DNA damage and a compromised DDR (i.e., slow growth, sensitivity to DNA damage and DNA strand breaks). Additionally, Alkbh8⁻/⁻ cells have elevated intracellular reactive oxygen species (ROS) and an increased ROS stress response, which can be attributed to defective ROS detoxification through attenuated GPX protein expression. These findings support a role for Alkbh8 in mammalian DNA damage and oxidative stress responses and implicate Alkbh8 in various cyto-protective processes like genome maintenance, cancer suppression, and protection against aberrant ROS production.
POSTERS

BIOCHEMICAL CHARACTERIZATION AND CRYSTALLIZATION OF THE DBF4-RAD53 COMPLEX
Almawi, Ahmad W. 1, Lindsay A. Matthews1, Darryl R. Jones2, Bernard P. Duncker2 and Alba Guarnè1
1Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON. L8S 4K1, Canada and the 2Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, Canada.

INVESTIGATING THE ROLE OF MLH3 IN MEIOTIC CROSSOVER MECHANISMS
Al-Sweel, Najla, Luigi Di Vietro, and Eric Alani
Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY

COORDINATION OF DNA REPLICATION INITIATION AND TRANSCRIPTIONAL REGULATION BY ESCHERICHIA COLI BETA SLIDING CLAMP
Babu, Vignesh M.P. and Mark D. Sutton
Department of Biochemistry, State University of New York at Buffalo

CONTRIBUTION OF MMR TO DAMAGE INDUCED MUTAGENESIS IN ESCHERICHIA COLI
Baron, Christopher M. and Mark D. Sutton
Department of Biochemistry, State University of New York at Buffalo

YRRC IS A MULTIFUNCTIONAL DNA REPAIR HELICASE IN BACILLUS SUBLTILIS
Bolz, Samantha A., Brian W. Walsh and Lyle A. Simmons,
Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, 48109 U.S.A.

PCH2 IS A HEXAMERIC RING ATPASE THAT REMODELS THE MEIOTIC CHROMOSOME AXIS PROTEIN HOP1
Chen, Cheng1, Ahmad Jomaa2, Tim West1, Joaquin Ortega2 and Eric Alani1
1Molecular Biology and Genetics, Cornell University, Ithaca, New York
2Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario

A COMPARATIVE ANALYSIS OF THE PRO-MUTAGENIC ACTIVITY OF HIGH-FIDELITY AND SPECIALIZED DNA POLYMERASES DURING THE REPLICATION OF OXIDIZED DNA LESIONS
Choi, Jung-Suk a,b, Anvesh Dasari a, Sneha Jukanti a, Mark Sutton c, Stephen J. Benkovic d, and Anthony J. Berdis a, b
a Department of Chemistry and the bCenter for Gene Regulation in Health and Disease, Cleveland State University, 2121 Euclid Avenue, Cleveland, OH 44115
B Department of Biochemistry, University of Buffalo, Buffalo, NY 14214
D Department of Chemistry, The Pennsylvania State University, University Park, PA 16802
IS THE AUTOPHAGIC DEGRADATION OF RNR1 REGULATED BY POST TRANSLATIONAL MODIFICATIONS?
Danon, Tamir, Michael Fasullo, Thomas Begley
College of Nanoscale Science and Engineering, University at Albany, SUNY, Albany, NY

DETERMINANT OF THE DNA BINDING SPECIFICITY OF THE ORIGIN RECOGNITION COMPLEX
Dowicki, Michael W., Prof. Bik Tye
Department of Molecular Biology and Genetics, Cornell University

IDENTIFICATION OF RAD5 REGULATORS DURING THE S. CEREVISIAE DNA REPLICATION STRESS RESPONSE
Gallo, David W. and Grant W. Brown.
Department of Biochemistry and Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada

A HIGH-THROUGHPUT SCREEN FOR NOVEL GENES INVOLVED IN MAINTAINING GENOME STABILITY IN S. CEREVISIAE
Hendry, Jason A., Jiongwen Ou and Grant W. Brown
University of Toronto

MULTIPLEXED QUANTIFICATION OF DNA DAMAGE RESPONSE PROTEINS
Hsieh, Yi-Ching, Ulrike Begley and Thomas J. Begley
College of Nanoscale Science and Engineering, University at Albany, Albany, New York 12203

GENOME-WIDE EFFECTS OF MINI-CHROMOSOME MAINTENANCE PROTEIN 2 DEFICIENCY ON ORIGINS OF DNA REPLICATION DEFINED BY CAPTURE AND RELEASE OF NASCENT STRANDS
Kunnev, Dimiter, Amy Freeland, Robert W. Leach and Steven C. Pruitt
Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, USA

TRAPPING AND VISUALIZING INTERMEDIATE STEPS IN THE MISMATCH REPAIR PATHWAY IN VIVO
Lenhart, Justin S., 1§, Monica C. Pillon2§, Alba Guarné2 and Lyle A. Simmons1*
1 Department of Molecular, Cellular and Developmental Biology
University of Michigan, 830 North University Ave, Ann Arbor, MI 48109-1048
2 Department of Biochemistry and Biomedical Sciences, McMaster University, 1280 Main Street, West, Hamilton, Ontario L8S 4K1

GENOMIC INSTABILITY IN KLF4 NULL MOUSE EMBRYONIC FIBROBLASTS INDUCES PREMATURE SENESCENCE
Liu, Changchang and Engda Hagos
Colgate University, Hamilton, NY
INVESTIGATING THE ROLE OF RFC-DNA CONTACTS IN THE PCNA CLAMP LOADING PATHWAY
Liu, Juan, Yayan Zhou, Manju Hingorani
Department of Molecular Biology and Biochemistry, Wesleyan University Middletown, CT

PROTECTION AGAINST DIETARY FAT-INDUCED DNA DAMAGE BY THE FANCONI ANEMIA PATHWAY
Moore, Elizabeth S., Erin K. Daugherity, David I. Karambizi, Teresa L. Southard, Robert S. Weiss
Center for Animal Resources and Education, and Department of Biomedical Sciences, Cornell University, Ithaca, NY

REPAIR OF INDUCED DOUBLE-STRAND BREAKS IN MITOCHONDRIAL DNA
Nagarajan, Prabha, Alexis Stein, Elaine Sia
Department of Biology, University of Rochester

CHARACTERIZATION OF NUCLEOID-ASSOCIATED PROTEIN sIHF
Nanji, Tamiza, Swierzcz, Julia, Gloyd, Melanie, Elliot, Marie and Guarné, Alba
Department of Biochemistry and Biomedical Sciences, and Institute for Infectious Disease Research, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4K1

MECHANISM OF TRANPOSITION TARGETING IN A TN7 RELATED TRANSPONSON
Petassi, Michael and Joe Peters
Department of Microbiology, Cornell University, Ithaca NY 14853

INVESTIGATING HOW THE DNA DAMAGE RESPONSE AND STEM CELL PROPERTIES OF TESTICULAR GERM CELL CANCERS AFFECT THEIR SENSITIVITY TO CHEMOTHERAPEUTICS
Department of Biomedical Sciences, Cornell University, Ithaca, New York 14853

CHARACTERIZATION OF THE DEFECTS IN THE ATP LID OF E. COLI MUTL THAT CAUSE TRANSIENT HYPERMUTABILITY
Pillon, Monica, Michelle Dubinsky, Randal N. Johnston, Shu-Lin Liu, and Alba Guarné
Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, L8S 4K1, Canada. Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB T2N 4N1, Canada.

GENOMIC QUALITY CONTROL IN MAMMALIAN OOCYTES
Rinaldi, Vera D., Ewelina Bolcun-Filas and John C. Schimenti
Department of Biomedical Sciences, Cornell University, Ithaca, NY. Center for Vertebrate Genomics, Cornell University, Ithaca, NY.
MISMATCH DETECTION BY MUTS OCCURS AT THE SITE OF DNA SYNTHESIS
Simmons, Lyle A. 1*, Jeremy W. Schroeder1, Yi Liao2, Julie S. Biteen3,
1Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, 48109 U.S.A.
2Department of Chemistry, University of Michigan

REGULATION AND KINETICS OF DNA DAMAGE INDUCED PROTEIN
RELOCALIZATION IN S. CEREVISIAE
Torres, Nikko P. and Grant W. Brown.
Department of Biochemistry and Donnelly Centre, University of Toronto.

HIGH-THROUGHPUT SCREENING OF DNA2 REGULATORS IN YEAST DNA
DAMAGE SIGNALING PATHWAY
Yimit, Askar and Grant W. Brown
Department of Biochemistry and Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, 160 College Street, Toronto, ON M5S 3E1, Canada

PURIFICATION AND ANALYSIS OF S. CEREVISIAE MSH2-MSH3
Yu, Xiaoyu, Shreya Sawant, Manju Hingorani: Wesleyan University, Middletown, CT 06459,
Department of Molecular Biology and Biochemistry, Wesleyan University, Middletown, CT
The process of DNA replication starts with the assembly of the pre-replication complex (pre-RC) at origins of replication. The activation and licensing of the pre-RC to define a functional replication fork is a multi-step process orchestrated by several cell cycle kinases. One of these key proteins is the *Saccharomyces cerevisiae* Dbf4-dependent kinase (DDK) complex, a heterodimer composed of regulatory subunit Dbf4 and catalytic subunit Cdc7, which phosphorylates targets found at licensed origins, hence triggering the onset of DNA replication. Additionally, DDK plays an important role in replication checkpoint responses. During genotoxic stress, DDK phosphorylates the checkpoint kinase, Rad53, thus leading to its full activation. Dbf4 is then phosphorylated in a Rad53-dependent manner, thereby inhibiting DDK activity. This, in turn, regulates DNA replication by suppressing the activation of unfired origins. Although the X-ray structures of the domains of Dbf4 and Rad53 mediating this interaction are known, it remains unclear how they interact. Based on previous biochemical and structural data, we have generated a model for the complex that shows good surface and charge complementarity. One characteristic of this model is that the N-terminus of Rad53 is spatially close to the C-terminus of Dbf4. To probe the validity of our model, we exploited this feature to produce the two proteins of the complex as a single polypeptide chain. This Rad53-Dbf4 fusion protein can be purified to homogeneity and is stable in solution. Using sparse matrix crystallization screens, we have obtained preliminary crystals of the Rad53-Dbf4 fusion and crystal optimization is currently underway. Beyond unveiling how the two proteins interact at a molecular level, this structure will demonstrate how molecular modeling coupled with biochemical characterization of protein interfaces can be exploited to stabilize transient complexes. Furthermore, DDK activity is upregulated in multiple cancers, and consequently Cdc7 has recently gained attention as an anti-cancer target. Identifying the interface on Dbf4 that renders the DDK complex inactive will be relevant for such developments.
INVESTIGATING THE ROLE OF MLH3 IN MEIOTIC CROSSOVER MECHANISMS

Al-Sweel, Najla, Luigi Di Vietro, and Eric Alani

Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY

In most organisms, correct chromosome segregation at the first meiotic division requires reciprocal exchange between homologs. Failure to achieve at least one crossover per homolog pair results in non-disjunction and aneuploid gametes. The process by which crossing over occurs is not yet well understood at a mechanistic level. The classic double strand break repair model proposes the induction of double-strand breaks followed by the formation of joint molecules which are then resolved as crossovers (Szostak et al. Cell 33:25). In most eukaryotes, the major crossover pathway involves the MutS mismatch repair (MMR) homologs MSH4-MSH5 and the MutL homologs MLH1-MLH3. Our lab and others hypothesize that MSH4-MSH5 acts to recognize and recruit MLH1-MLH3 endonuclease activity to joint molecule intermediates which are then resolved as crossover products (Nishant et al. Genetics 179:747, Snowden et al. Mol Cell 15:437, Zakharyevich et al. Cell 149:334). MLH3 protein has been shown to play a minor role in MMR in addition to its role in meiosis (Flores-Rozas and Kolodner PNAS 95:12404). It has a highly conserved endonuclease domain, and point mutations in this domain conferred null-like defects in both MMR and crossing over (Nishant et al. Genetics 179:747).

We have performed an alanine scanning mutagenesis of MLH3 in which 56 mutant alleles have been generated. The mutants are being analyzed for their MMR and crossover functions in the model organism Saccharomyces cerevisiae. We are specifically interested in determining whether the MMR and meiotic functions of MLH3 are genetically separable. I will present some mlh3 mutations that appear to confer such phenotypes. The comprehensive analysis of such features will fill the gaps of the crossover pathway at a mechanistic level and provide a tool for recombination studies in higher organisms.
COORDINATION OF DNA REPLICATION INITIATION AND TRANSCRIPTIONAL REGULATION BY ESCHERICHIA COLI BETA SLIDING CLAMP

Babu Vignesh M.P. and Mark D. Sutton
Department of Biochemistry, State University of New York at Buffalo

The beta sliding clamp, encoded by the dnaN gene, is known to interact with several proteins that are required for DNA replication and repair in Escherichia coli. These interactions are conserved across all domains of life. Beta clamp is also implicated in the regulation of polymerase access to the replication fork. The polymerases are considered to be competitive in gaining access to the cleft region of the clamp and the regulation of their concentration in turn regulates this process. In eukaryotes, beta clamp undergoes post translational modifications that regulate the access of polymerases involved in translesion synthesis (TLS). This work aims at understanding the role of beta clamp interactions outside of the cleft that might play a role in regulation of partner access to the DNA. We have identified a group of residues in the beta clamp that are required for the cell survival in the presence of Nitrofurazone (NFZ) and methyl methanesulfonate (MMS). In contrast, these residues are not required for survival after UV treatment. We also demonstrate that a point mutant strain dnaN\textsubscript{E202K} that failed to confer the cold sensitivity of umuDC overexpression is sensitive to NFZ and MMS but is not epistatic with dinB (Pol IV). Hda, a homolog of DNA initiator protein DnaA, functions in regulating DnaA-ATP to DnaA-ADP ratio by a process known as Regulatory inactivation of DnaA (RIDA). Our results suggests that the dnaN\textsubscript{E202K} strain mimicks overproduction of Hda resulting in reduced number of initiation events. We also show that the E202K mutation results in the modulation of the transcriptional network as a response to replication defect indicating that there is tight coordination between the two processes. This project further aims to characterize the identified mutants genetically and biochemically to understand mechanisms underlying regulation of DNA replication by beta clamp.
CONTRIBUTION OF MMR TO DAMAGE INDUCED MUTAGENESIS IN ESCHERICHIA COLI

Baron, Christopher M. and Mark D. Sutton

Department of Biochemistry, State University of New York at Buffalo

Mismatch repair (MMR) is a repair pathway that acts to ensure genomic fidelity by recognizing and excising errors caused during replication. The result is a single strand DNA gap that is filled by a DNA polymerase, ideally, in an error free manner. Escherichia coli possesses 3 lower fidelity polymerases which are known as Translesion synthesis (TLS) polymerases. These polymerases are regulated by a damage response known as the SOS response, a system which senses DNA damage and upregulates over 40 genes involved in repair of DNA damage. One TLS polymerase, Pol V, is responsible for most mutagenesis catalyzed during the SOS response. I hypothesize that that during the SOS response, MMR facilitates SOS mutagenesis by generating a gap that Pol V gains access and catalyzes error prone synthesis. To this end, I have demonstrated that mutations induced by Hydrogen peroxide, Ultra violet radiation and Methyl methanesulfonate requires both MMR protein and activity. Utilizing a construct that constitutively expresses Pol V, I have also demonstrated that mutagenesis catalyzed by Pol V can be further enhanced by stimulation of MMR. I am currently investigating the impact MMR may have on the SOS response, which would influence the level of Pol V present in the cell. My results suggest that MMR facilitates mutagenesis by providing increased opportunity for Pol V to gain access to the DNA template. Future efforts will determine whether loss of MMR affects levels of available Pol V within the cell.
**YRRC IS A MULTIFUNCTIONAL DNA REPAIR HELICASE IN *BACILLUS SUBTILIS***

Bolz, Samantha A., Brian W. Walsh and Lyle A. Simmons

Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, 48109 U.S.A.

DNA repair is critical for maintaining the integrity of genomic DNA in organisms from bacteria to humans. RecD2, is a helicase that has been shown to be important for mismatch repair in the bacterium *Bacillus anthracis*. Here, we tested the RecD2 helicase YrrC for a role in genome maintenance in *Bacillus subtilis*. We show that deletion of yrrC confers a modest increase in spontaneous mutagenesis as measured by rifampicin and trimethoprim resistance. These results indicate that YrrC contributes to the efficiency of the mismatch repair pathway in *B. subtilis* although mismatch repair is not dependent on YrrC function. To further characterize YrrC, we tested the deletion strain for sensitivity to several different DNA damaging conditions including: MMS, mitomycin C, phleomycin, and UV to determine if YrrC plays a role in repair of these lesions. Our current results show that loss of YrrC causes a substantial sensitivity to each of these treatments with the exception of UV. With these results we show that YrrC functions in several aspects of DNA repair and is critical for genome maintenance in *B. subtilis*. 
PCH2 IS A HEXAMERIC RING ATPASE THAT REMODELS THE MEIOTIC CHROMOSOME AXIS PROTEIN HOP1

Chen, Cheng\textsuperscript{1}, Ahmad Jomaa\textsuperscript{2}, Tim West\textsuperscript{1}, Joaquin Ortega\textsuperscript{2} and Eric Alani\textsuperscript{1}
\textsuperscript{1}Molecular Biology and Genetics, Cornell University, Ithaca, New York
\textsuperscript{2}Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario

The Pch2 protein of budding yeast is required for proper meiotic chromosome axis structure by maintaining the domainal organization of the synaptonemal complex proteins Hop1 and Zip1. It regulates meiotic DSB repair outcomes to favor recombination between homologs, is important for the progression of meiotic recombination, and maintains ribosomal DNA stability. Pch2 homologs are present in fruit flies, worms and mammals, but the biochemical functions of these proteins are unknown. We show that: 1. Pch2 is an ATPase that oligomerizes into single hexameric rings in the presence of nucleotides. 2. Pch2 binds to Hop1, an axial component of the synaptonemal complex that is critical to establish interhomolog repair bias. 3. Pch2 displaces Hop1 from a large DNA substrate. 4. Both ATP binding and hydrolysis by Pch2 are required for Pch2-Hop1 transactions. Based on these and previous cell biological observations, we suggest that Pch2 impacts meiotic chromosome function by directly regulating Hop1 localization.
A COMPARATIVE ANALYSIS OF THE PRO-MUTAGENIC ACTIVITY OF HIGH-FIDELITY AND SPECIALIZED DNA POLYMERASES DURING THE REPLICATION OF OXIDIZED DNA LESIONS

Choi, Jung-Suk\textsuperscript{a,b}, Anvesh Dasari\textsuperscript{a}, Sneha Jukanti\textsuperscript{a}, Mark Sutton\textsuperscript{c}, Stephen J. Benkovic\textsuperscript{d}, and Anthony J. Berdis\textsuperscript{a,b}

\textsuperscript{a}Department of Chemistry and the \textsuperscript{b}Center for Gene Regulation in Health and Disease, Cleveland State University, 2121 Euclid Avenue, Cleveland, OH 44115
\textsuperscript{c}Department of Biochemistry, University of Buffalo, Buffalo, NY 14214
\textsuperscript{d}Department of Chemistry, The Pennsylvania State University, University Park, PA 16802

The oxygen-rich environment present in the lung can create various pro-mutagenic DNA lesions such as 8-oxoguanine, thymine glycol, and 2,6-diamino-4-hydroxy-5-formamidopyrimidine. Each DNA lesion can be misreplicated in a complex biological process known as transl lesion DNA synthesis (TLS) which can cause genetic diseases such as lung cancer or enhance the development of drug resistance in pathogenic microorganisms that infect the lung. In this report, we evaluate the pro-mutagenic behavior of two commonly formed oxidized lesions, 8-oxoguanine (8-OG), and thymine glycol (TG), by quantifying the ability of high-fidelity and specialized DNA polymerases to replicate each DNA lesion. During normal DNA synthesis, high-fidelity polymerases (eukaryotic pol\(\delta\) and the bacteriophage T4 DNA polymerase) show remarkable fidelity by exclusively incorporating dCTP opposite a template G. However, both polymerases display pro-mutagenic tendencies when replicating 8-OG as dATP is efficiently inserted opposite the oxidized lesion. In this case, pol\(\delta\) possesses higher fidelity than the T4 polymerase as it shows a 10-fold higher efficiency for “error-free” insertion of dCTP rather than “error-prone” incorporation of dATP opposite 8-OG. The specialized eukaryotic DNA polymerase, pol\(\eta\), shows less fidelity when replicating undamaged DNA. Remarkably, pol\(\eta\) shows unusual fidelity as it catalyzes a higher degree of “error-free” synthesis of 8-OG compared to pol\(\delta\) or the bacteriophage T4 polymerase. In addition, pol\(\eta\) is the only of four different DNA polymerases tested that efficiently incorporates nucleotides opposite the oxidized pyrimidine, TG. However, this lesion is replicated in an “error-prone” manner as dTTP is inserted as efficiently as dATP. Collectively, these kinetic data present a complex picture for the mutagenic potential of oxidized DNA lesions. In the case of 8-OG, high-fidelity DNA polymerases appear more likely to perform “error-prone” replication compared to the specialized DNA polymerase, pol\(\eta\). In contrast, TG behaves as a strong replicative block to high fidelity DNA polymerase such as pol\(\delta\) and as a pro-mutagenic lesion by pol\(\eta\). Studies are currently evaluating the ability of these and other specialized DNA polymerases to replicate other oxidized DNA lesions in order to develop a more comprehensive understanding of TLS and mutagenesis.
IS THE AUTOPHAGIC DEGRADATION OF RNR1 REGULATED BY POST
TRANSLATIONAL MODIFICATIONS?

Danon, Tamir, Michael Fasullo, Thomas Begley
College of Nanoscale Science and Engineering, University at Albany, SUNY
Albany, NY

The Ribonucleotide Reductase Complex (RNR) is the rate-limiting step in the production of dNTPs and is heavily regulated in response to DNA damage. The complex is a tetramer of 2 major (Rnr1-Rnr1 or Rnr1-Rnr3) and 2 minor (Rnr2 and Rnr4) subunits. Recently it was shown that Rnr1 soluble protein levels are regulated by autophagy, which targets Rnr1 to the vacuole for degradation. Using a GFP tag we have reported the increase in Rnr1-GFP foci in response to methyl methanesulfonate (MMS) damage and in response to Target Of Rapamycin (TOR) inhibition by rapamycin. Using autophagy deficient mutants, we supported the idea that these foci are found in autophagosomes. In contrast, Rnr3-which exhibits high protein sequence similarity to Rnr1, does not form foci in response to DNA Damage or TOR inhibition. We have identified a putative acetylation site at K853 in Rnr1 that is not found in Rnr3, and postulated that K853 is post translationally modified to regulate the specific autophagy of Rnr1. We mutated K853 to form the mutants Rnr1-K853Q and Rnr1-K853A. Relative to wild-type Rnr1, we have shown that Rnr1-K853Q containing cells display increased sensitivity to MMS-suggesting lower Rnr1 soluble protein levels. In contrast, Rnr1-K853A display increased resistance to MMS-suggesting higher Rnr1 soluble protein levels. Compared to wild-type Rnr1-GFP, both mutants display significantly altered foci dynamics in response to MMS damage. Specifically, Rnr1-K853Q showed a dampened increase in MMS-induced foci compared with wild-type, while Rnr1-K853A displayed no increase in foci in response to MMS. Our data supports the notion that acetylation-or another post translational modification (PTM) - regulates Rnr1 degradation in response to DNA damage. The goal of this project is to identify the molecular mechanism of Rnr1 specific autophagy, as autophagy is a process whose malfunction in humans is implicated in numerous disease states, from cancer to obesity. Also, molecular targeting via acetylation could define a new type of autophagy. We will continue to investigate the PTMs involved in Rnr1 degradation, with plans to uncover the mechanism of this protein targeting by the autophagy machinery.
DETERMINANT OF THE DNA BINDING SPECIFICITY OF THE ORIGIN RECOGNITION COMPLEX

Dowicki, Michael W., Prof. Bik Tye

Department of Molecular Biology and Genetics, Cornell University

The process of DNA replication is regulated to ensure that the entire genome is replicated only once during a cell cycle. Eukaryotic DNA replication begins with the binding of the Origin Replication Complex (ORC) to multiple replication origins or Autonomously Replicating Sequences (ARSs) on each chromosome. The ORC machinery is conserved from fungal to mammalian systems, however the ARSs to which the ORC binds have diverged significantly. In the budding yeast S. cerevisiae the ORC binds a well-defined 17bp ARS consensus sequence, conversely in the fission yeast S. pombe ORC binds to ill-defined AT rich sequences in a stochastic manner.

Recently the replication origins of the yeast K. lactis have been identified as a 50bp sequence necessary and largely sufficient for replication. Through testing of plasmids constructed to contain either S. cerevisiae or K. lactis ARSs, it was found that each species is largely unable to replicate ARSs from the other species, indicating that the replication machinery has significantly diverged in each to specifically recognize its own origin sequence. In this study, I am examining how subunits of the ORC complex determine the binding specificity of the ORC complex.

The ORC proteins contain the DNA binding AAA and WHD domains in their C-termini. I have constructed S. cerevisiae strains containing chimeric ORC subunits that interact with the S. cerevisiae machinery while containing the K. lactis DNA binding domains. So far, I have constructed several strains that contain integrated chimeras of varying lengths of Orc4 and Orc5. None of them were able to substitute the endogenous ORC subunit. However, several chimeric ORC constructs resulted in a loss of ARS dependent silencing at the HMR locus, suggesting that the chimeric constructs have altered the binding specificity of the ORC complex. Using Orc6 in a ChIP-seq experiment, I am analyzing the binding of the chimeric ORC complex. The binding of ORC at the ARS locus responsible for the silencing of the reporter in the ARS dependent silencing region has been noticeably changed, suggesting that the loss of ARS dependent silencing is due to a change in the binding of the ORC complex. Additionally, unique binding signals corresponding to sites bound by the chimeric ORC complex are observed at about a dozen intergenic regions.
IDENTIFICATION OF RAD5 REGULATORS DURING THE S. CEREVISIAE DNA REPLICATION STRESS RESPONSE

Gallo, David W. and Grant W. Brown.
Department of Biochemistry and Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada

We recently identified the complement of proteins that change intracellular localization during replication stress. Of these 23 form sub-nuclear foci, one of which is Rad5. Rad5 is in the PRR pathway that signals to promote replication bypass of blockages. In the current study we aim to define the mechanisms by which Rad5 is recruited to nuclear foci during replication stress. Rad5 foci form predominantly during S phase, suggesting that these could be sites of replication repair. We probed the role of PRR genes and found that RAD18, RAD6, and MMS2 are required for Rad5 focus formation. We then screened the yeast gene deletion collection by high-throughput confocal microscopy and identified 23 additional genes that are important for Rad5 focus formation during HU-induced replication stress. Genetic interaction analysis of these 23 gene deletion mutants in a RAD5 deletion background and in the presence of HU revealed 5 epistatic and 10 additive interactions. We are currently characterizing potential regulators of Rad5, and identifying additional protein components of Rad5 foci to understand how Rad5 foci function in the cellular DNA replication stress response.
Cells respond to DNA damage by evoking the DNA damage response (DDR) pathway – a highly conserved kinase cascade that promotes cell-cycle arrest and the upregulation of genes necessary for efficient repair. The ribonucleotide reductase (RNR) complex is a well-studied transcriptional target of the DDR. RNR catalyzes the rate-limiting step of dNTP synthesis and its induction in response to damage enables increased dNTP pools required for repair. Notably, the alternative large subunit of RNR, RNR3, has a sharply delineated transcriptional profile in response to DNA damage, with its mRNA levels increasing by upwards of 100-fold.

We are using RNR3’s unique expression profile to screen for gene deletion mutants that result in constitutive DDR activation, which is an indicator of genome instability. To this end, we are employing the Reporter Synthetic Genetic Array (R-SGA) high-throughput screening methodology. R-SGA utilizes the S. cerevisiae non-essential deletion collection, in combination with automated yeast genetics and a fluorescent protein reporter system, to assay the effect of single gene knockouts on the expression of a gene of interest in a high-throughput format. Briefly, a strain harboring Rnr3-GFP and a constitutively expressed RFP was crossed with the entire non-essential deletion collection using the SGA methodology. The collection was subjected to fluorescence microscopy and Rnr3 abundance was scored in each deletion as the $\log_2(\text{GFP/RFP})$ ratio.

We identified 136 deletion mutants with increased Rnr3 abundance in untreated conditions ($Z$-score > 2). Gene Ontology process enrichment revealed three primary functional associations: (1) transcriptional regulation, (2) DNA repair and (3) DNA replication. At the level of transcription, we identified known regulators of RNR3, such as the DNA damage responsive transcriptional repressor RFX1 and the co-repressor complex ITC1/ISW2. Deletion mutants associated with DNA repair included those functioning in double-stranded break repair, such as members of the RAD52 epistasis group ($\text{RAD51, RAD52, RAD54 and RAD55}$) and SGS1, which resolves double-strand break repair intermediates. Deletion mutants associated with DNA replication tended to be involved in maintaining replication fork stability and included MRC1, which is a member of the fork pausing complex, and POL32, a subunit of Pol $\delta$ that increases its affinity to the sliding clamp.

Future work will be focused on probing mechanisms maintaining genome stability in the context of diverse DNA damage events, induced by agents such as methyl methanesulfonate, camptothecin and cisplatin. A systematic assay of Rnr3 abundance across a variety of damage types could provide valuable insights into damage-specific repair mechanisms as well as facilitate the discovery of novel genes involved in maintaining genome stability.
MULTIPLEXED QUANTIFICATION OF DNA DAMAGE RESPONSE PROTEINS

Hsieh, Yi-Ching , Ulrike Begley and Thomas J. Begley

College of Nanoscale Science and Engineering, University at Albany, Albany, New York 12203, USA.

DNA damage has been implicated as a critical factor of both aging and cancer development. UV from sunlight, radiation from the environment and medical computerized tomography (CT), chemical mutagens and endogenous metabolic reactions all can cause cellular DNA damage. To maintain the integrity of DNA, there is a DNA damage response pathway which is comprised of damage sensors, signal transducers and repair machines. However, each individual presents differences in their response to DNA damage, meaning the ability to effectively repair DNA damage after exposure to genotoxicants varies from person to person. The ability to establish a method for the measurement of DNA repair capacity would be very useful to guide lifestyle or clinical procedure decisions. We have developed multiplexed protein quantification assays to measure the levels and post-translational modification status of DNA damage response proteins using the MesoScale Discoveries (MSD) platform. The MSD system is a high throughput system, based on sandwich-ELISA technology with electro-chemiluminescence detection. We have developed five working assays to quantitatively measure the molecular levels of ATM and CHK2, phosphorylated status of ATM (S1981), CHK2 (T68) and p53 (S15). These assays had been employed to measure the cellular response to ionizing radiation or DNA-damaging chemicals (bleomycin, etoposide and hydrogen peroxide) in HEK293, a spectrum of breast cancer and normal breast cell lines, purified human peripheral blood mononuclear cells (PBMCs) and CAM (chick chorioallantoic membrane) xenograft tumors. Proof of DNA double strand breaks was monitored by H2AX staining, an initial indicator of the DNA damage response. By using our MSD assays, we can measure changes in protein or post translational modification levels up to 25-fold compared to untreated control. With as low as 1.25 g of irradiated lysates, we can detect ~3 fold increases in p-p53S15 and ~2 fold increases in CHK2 assays, respectively. Moreover, using our CHK2 assay we measured a ~14 fold increased signal in HEK-239G cells after 1 Gy ionizing radiation exposure. Similar results were demonstrated in breast cancer cell lines. Using immunoblots, MDA-MB231 cells have negligible expression levels of CHK2 and phosphorylated CHK2 at T68 with or without ionizing radiation. Using the same batch of lysates, our CHK2 and p-CHK2T68 MSD assays measured ~3 and ~1.5 fold increased signal compared to control, respectively. Our developed assays were also used to measure DNA damage signals in vivo, using xenograft based CAM tumors. This new technology allows us to quantify the initial component of an individual’s DNA repair capacity in a high throughput manner and will be applied to clinical samples in a future study.
Mice that are deficient for mini-chromosome maintenance protein (Mcm) expression or function exhibit elevated levels of genome instability and high rates of cancer. Mcm proteins function as the replicative helicase and are loaded onto chromatin during G1 phase of the cell cycle as part of the DNA replication licensing complex. To determine if Mcm deficiency alters the sites at which DNA replication initiates, nascent strands in WT and Mcm2 deficient mouse embryonic fibroblasts were mapped, genome wide, using a method of nascent strand capture and release. Here we show that Mcm2 deficiency results in a decreased density of nascent strands in early replicating regions of the genome.
TRAPPING AND VISUALIZING INTERMEDIATE STEPS IN THE MISMATCH REPAIR PATHWAY IN VIVO

Lenhart, Justin S. 1§, Monica C. Pillon2§, Alba Guarné2 and Lyle A. Simmons1* 14

1 Department of Molecular, Cellular and Developmental Biology
University of Michigan, 830 North University Ave, Ann Arbor, MI 48109-1048
2 Department of Biochemistry and Biomedical Sciences, McMaster University, 1280 Main Street, West, Hamilton, Ontario L8S 4K1

MutS and MutL are highly conserved proteins required for mismatch repair in organisms ranging from bacteria to humans. MutS is responsible for mismatch detection and the recruitment of MutL to mismatch proximal DNA through a mechanism that is unknown in most organisms. Here, we used peptide array mapping and chemical crosslinking experiments to identify the discrete site on MutS that is occupied by MutL. We identified two adjacent phenylalanine residues, F319 and F320, located in an unstructured loop of MutS that facilitate MutL interaction. Disruption of this site renders MutS defective in binding MutL in vitro and broken for recruitment of MutL in vivo, while also eliminating mismatch repair in vivo. Analysis of MutS repair complexes in live cells shows that MutS mutants defective in interaction with MutL are trapped in an unregulated loading cycle in response to mismatch detection. Furthermore, these mutant MutS repair complexes persist on DNA away from the DNA polymerase, suggesting that MutS remains loaded on mismatch proximal DNA awaiting arrival of MutL. Upon arrival of MutL, MutS-DNA bound complexes require a step of repair after MutL recruitment to stimulate disassembly. Furthermore, we present evidence that mismatch excision signals disassembly of MutS complexes in vivo. We also provide evidence showing that MutS and MutL interact independent of mismatch binding by MutS in vivo and in vitro. This finding suggests that MutL transiently probes MutS to determine if MutS is mismatch bound before initiating downstream repair events. Together, these data provide novel insights into the mechanism that MutS employs to recruit MutL, and the consequences that ensue when MutL recruitment fails during mismatch repair in living cells.

§ These authors contributed equally
GENOMIC INSTABILITY IN KLF4 NULL MOUSE EMBRYONIC FIBROBLASTS INDUCES PREMATURE SENESCENCE

Liu, Changchang and Engda Hagos
Colgate University, Hamilton, NY

BACKGROUND: Cellular senescence is a potent anti-cancer mechanism whereby cells that are damaged or otherwise at risk for cellular transformation are triggered to irreversibly lose the ability to undergo cell proliferation. Understanding the pathways that lead to the onset of senescence could prove useful in the development of cancer treatments. Kruppel-like factor 4 (KLF4) is a transcription factor that has been implicated in the development of cancer. It has a number of tumor suppressor functions, such as the ability to mediate p53 transactivation of p21WAF1/Cip1 to trigger cell cycle arrest. In our previous work we have shown that Klf4 null MEFs exhibit increased genomic instability as compared to the wildtype MEFs. Further, it has been observed that Klf4 null MEFs express increased amounts of p21 and enter senescence prematurely.

AIM: In this study we aim to determine whether the increased genomic instability in KLF4-null MEFs is responsible for those cells to enter premature senescence.

METHODS: Mice heterozygous for the Klf4 alleles (Klf4+/−) on a C57BL/6 background were crossbred. MEFs that are wild type (Klf4+/+) or null (Klf4−/−) for the Klf4 alleles were derived from day-13.5 embryos. Pre-senescent MEFs were passed every three days and counted until the reach senescence. Population doubling was calculated to determine levels of cell proliferation. Western Blotting was performed to determine the level of cell-cycle regulatory proteins such as p21 and p53. The presence of cellular senescence was determined and quantified by Senescence-associated β-galactosidase staining. Immunostaining against γH2AX was performed to determine the amount DNA damage. Wildtype MEFs were treated with Nutlin-3 or doxorubicin to induce early senescence.

RESULTS: KLF4-null MEFs enter senescence much earlier than wildtype MEFs due to the accumulation of p21 in p53 dependent manner. Klf4 null MEFs also show increased γH2AX foci as compared to wildtype. Furthermore, wildtype cells treated with Nutlin-3, a drug that upregulated p53, promotes an early entrance into senescence.

CONCLUSIONS: Results of this study demonstrate that Klf4 null mouse embryonic fibroblasts exhibit higher levels of genomic instability and senescent prematurely in p53 dependent manner when compared to wild-type MEFs. These results indicate that Klf4 plays a crucial role in maintaining genetic stability and support the previous findings that Klf4 is a tumor suppressor.
INVESTIGATING THE ROLE OF RFC-DNA CONTACTS IN THE PCNA CLAMP LOADING PATHWAY
Liu, Juan, Yayan Zhou, Manju Hingorani
Department of Molecular Biology and Biochemistry, Wesleyan University
Middletown, CT 06459

Replicative DNA polymerases utilize a conserved sliding clamp mechanism for processive DNA synthesis. Clamps are ring shaped proteins that encircle and slide on DNA, thus serving as mobile tethers for DNA polymerases. These proteins are loaded onto DNA by clamp loaders in a reaction fueled by ATP. In S. cerevisiae, PCNA sliding clamp is loaded onto DNA by the multi-subunit Replication Factor C protein (RFC). Clamp loading is a complex multi-step catalytic reaction, minimally composed of: ATP binding to RFC – RFC binding and opening PCNA – DNA entering RFC-PCNA complex – DNA-induced rapid ATP hydrolysis – PCNA closure – PCNA*DNA release from RFC. Transient kinetic studies of primarily E. coli, S. cerevisiae, and T4 bacteriophage clamp loaders have provided detailed mechanistic information on this essential process in DNA replication, repair and recombination.

Here we describe an investigation of a new hypothesis about the clamp loader catalytic mechanism, based on crystal structures of the above model proteins (1). This “allosteric switch” hypothesis posits that—in the absence of DNA, the catalytic Walker B glutamate residue is held in an inactive conformation by a conserved basic residue (switch residue) within each RFC subunit; in the presence of DNA, the switch residue moves to bind the DNA phosphate backbone and releases the glutamate, which can then adopt an active conformation, stimulate ATP hydrolysis and complete the reaction. We are testing this hypothesis by kinetic analysis of single DNA binding/switch residue mutants of S. cerevisiae RFC subunits. Our goal is to determine exactly which step(s) in the reaction are regulated by these residues, and thus define their potentially critical role in the clamp loading mechanism.

PROTECTION AGAINST DIETARY FAT-INDUCED DNA DAMAGE BY THE FANCONI ANEMIA PATHWAY
Moore, Elizabeth S. 1,2, Erin K. Daugherity1, David I. Karambizi2, Teresa L. Southard2, Robert S. Weiss2
1Center for Animal Resources and Education, and 2Department of Biomedical Sciences, Cornell University, Ithaca, NY

Fanconi anemia (FA) is a genomic instability syndrome affecting 1 in 131,000 people in the United States. FA phenotypes include developmental defects, bone marrow failure, cancer predisposition, and metabolic disorders. The FA pathway becomes activated by DNA replication stresses and plays a major role in responding to interstrand DNA crosslinks (ICLs). Though it is known that FA patients are hypersensitive to exogenous genotoxins, the endogenous sources of damage repaired by the pathway remain poorly characterized. Our objective was to test the hypothesis that the Fanconi Anemia pathway protects against DNA damage caused by fatty acid metabolism using a mouse model based on targeted disruption of \textit{FancD2}, which encodes a central component of the pathway. \textit{Fancd2}\textsuperscript{-/-} and wildtype (WT) mice were either continued on standard diet (SD) or challenged with a high fat, high cholesterol diet (HFD) at weaning, which led to hepatic steatosis and hepatitis within weeks. A cohort of mice fed HFD for eight weeks displayed an increase in hepatic pathology, including bile duct hyperplasia, neutrophil infiltration, and lipogranuloma formation in \textit{Fancd2}\textsuperscript{-/-} mice as compared to WT controls. HFD feeding induced a greater elevation in expression of the p53 target gene \textit{p21} as measured by qPCR in \textit{Fancd2}\textsuperscript{-/-} mice compared to WT controls. This trend toward increased pathologic changes and a greater DNA damage response in \textit{Fancd2}\textsuperscript{-/-} mice supports the hypothesis that the FA pathway plays a role in protecting against HFD induced DNA damage. We further propose that ROS (reactive oxygen species) and lipid peroxidation products, which result in DNA damage, including ICLs, are a source of endogenous damage repaired by the FA pathway. We will test this hypothesis by determining how \textit{Fancd2} inactivation affects HFD-induced oxidative DNA damage and associated DNA damage responses. The endogenous stresses to which the FA pathway responds is a major unanswered question of fundamental significance to our understanding of genome maintenance and disease pathogenesis in FA patients. The \textit{Fancd2}-deficient mouse model described here displays hypersensitivity to HFD challenge and may be a powerful model for defining the roles of the FA pathway in protecting against HFD-induced DNA damage.
REPAIR OF INDUCED DOUBLE-STRAND BREAKS IN MITOCHONDRIAL DNA

Nagarajan, Prabha, Alexis Stein, Elaine Sia
Department of Biology, University of Rochester

Mitochondria contain an independently maintained genome that encodes several proteins required for cellular respiration. The current understanding of the maintenance and repair of mitochondrial DNA (mtDNA) is quite limited compared to our understanding of similar events in the nucleus. Due to its close proximity to reactive oxygen species, mtDNA is prone to a high mutation rate. Among different types of DNA lesions that can be produced, double-strand breaks (DSBs) can be extremely deleterious to mtDNA. Mutations in the mitochondrial genome have been linked to several human diseases including Kearns-Sayre and Pearson-Marrow syndromes as well as potentially contributing to diabetes, cancer, and aging related disorders. To maintain its genome integrity, many nuclear DNA repair proteins are now known to localize in mitochondria but their precise function and mechanism remains largely unknown. In our study, we use a mitochondrially targeted restriction endonuclease (KpnI) to produce a unique DSB in a synthetic mitochondrial Arg8 mutant gene that is flanked by 96 base pair direct repeats of the COX2 gene in Saccharomyces cerevisiae. Repair of this DSB leads to the loss of Arg8 mutant gene and gain of functional COX2 gene, producing respiring but arginine auxotrophic cells. The deletion of several known nuclear DSB repair proteins significantly impact the rate of these deletion events. Southern blot analysis of these strains indicates that the kinetics of repair are also altered.
CHARACTERIZATION OF NUCLEOID-ASSOCIATED PROTEIN sIHF

Nanji, Tamiza¹; Swiercz, Julia²; Gloyd, Melanie¹; Elliot, Marie² and Guarné, Alba¹

¹Department of Biochemistry and Biomedical Sciences, and ²Department of Biology and Institute for Infectious Disease Research, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4K1

Chromosomal DNA must be compacted to fit within the boundaries of the cell while being accessible for various cellular processes. Hence, organizing DNA effectively is central to the functioning of any cell. To surmount this challenge, bacteria employ multiple nucleoid-associated proteins that help compact the chromosome through bending, bridging, and wrapping DNA molecules. Here, we describe the characterization of a novel nucleoid-associated protein in Streptomyces coelicolor (sIHF). We have used a combination of genetics, cell biology, biochemistry, and structural biology to characterize the functions of sIHF in maintaining DNA topology. We found that sIHF localizes with the nucleoid and that sIHF knockout mutant strains displayed abnormal chromosome compaction. sIHF binds DNA in a sequence-independent, but length-dependent manner. The co-crystal structure of sIHF bound to DNA illustrates that sIHF contains a helix-two turns-helix (H2TH) motif and interacts simultaneously with two DNA molecules through distinct protein interfaces. H2TH motifs have been found in topoisomerase VI, ribosomal protein S13 and endonuclease VIII where they are presumed to enhance DNA binding. We have also shown that sIHF affects the activity of TopA, the only topoisomerase in Streptomyces coelicolor. Further biochemical characterization is ongoing to establish the relative contributions of the two DNA-binding interfaces of sIHF and to probe the interaction between sIHF and TopA. The fact that sIHF interacts with DNA through two distinct surfaces, suggests a probable role in bridging DNA fragments and provides a framework for further structure-function analysis of sIHF. Given that this protein is specific to actinomycetes, and that the Mycobacterium homolog of sIHF (mIHF) is essential for viability, our studies provide the groundwork to explore novel drug targets for Mycobacterium tuberculosis and Mycobacterium leprae.
MECHANISM OF TRANSPOSITION TARGETING IN A TN7 RELATED TRANSPONSON

Petassi, Michael and Joe Peters
Department of Microbiology, Cornell University, Ithaca NY 14853

The Peters Lab works primarily with a transposable element called Tn7. A transposable element, or transposon, is a sequence of DNA that can self-transpose itself to other pieces of DNA in a bacterial cell. In the standard Tn7 element, there are genes for five proteins that allow for transposition, these proteins are called TnsA, TnsB, TnsC, TnsD, and TnsE. The first three proteins, TnsABC, are the core machinery of transposition, while TnsD and E are transposition-directing proteins that determine where this transposition is directed. TnsD directs transposition into what has been named the attTn7 site, located downstream of the glmS gene, by recognizing a region found highly conserved among bacteria. TnsE directs transposition into bacterial plasmids capable of moving between cells by recognizing lagging strand DNA replication, which occurs during the process of transfer. This allows Tn7 elements to move to other bacterial strains. Besides these genes, the Tn7 element also contains a variable region that can contain different genes, such as antibiotic resistance genes.

Since Tn7 elements have pathways that allow them to transpose into both chromosomal DNA and plasmids, these elements can move very easily between different strains and even distantly related species. Tn7 elements have been found in many varying bacteria. These elements can also have antibiotic resistance genes and/or genes allowing the bacterial host to be pathogenic in their variable region. The ability for Tn7 to move among bacteria can allow for these genes to be moved between bacteria, resulting in dangerous or hard to treat human pathogens.

Recently an element was identified that is similar to Tn7 that will be referred to as Tn7-like. This element contains the TnsA, B, and C proteins, which are related to those found in Tn7, and also a TnsD' protein, which is similar to the Tn7 TnsD. However, these elements have not been found in the attTn7 site, so it is clear that TnsD' does not recognize a sequence in the glmS gene. This Tn-7 like element tends to be found in a site at the end of the yhiN gene in bacteria. This suggests that TnsD' may work in a way similar to TnsD but simply direct into a difference place in the chromosome. One of these yhiN insertions was observed by screening, further supporting this idea. Additionally, this Tn7-like element has also been found in several plasmids, suggesting that it is capable of targeting plasmids for transposition as well as the chromosomal site. Whether this is due to the TnsD' protein or some other factor in the transposon remains to be seen.

An in vivo system that allows for monitoring of transposition events has been created that will allow us to map where insertions are taking place. We are also looking at other possible factors that could influence the frequency of transposition, including the effect of the host background. We hope to determine if this TnsD' protein is targeting the yhiN site and what mechanism is responsible for the ability of this element to insert into plasmids.

This work was funded in part by the NSF MCB1244227.
Testicular germ cell cancers (TGCCs) are the most common malignancies of young men and are unusual in being highly responsive to conventional chemotherapies, even at advanced, metastatic stages. We hypothesize that unique DNA damage response (DDR) properties in the germ cells from which TGCCs arise underlie their increased responsiveness to genotoxic chemotherapies. Unlike most solid cancers, human TGCCs do not show DDR activation at early stages of tumorigenesis and rarely develop DDR gene mutations. To investigate how DDR mechanisms contribute to the treatment sensitivity of TGCCs, we generated a novel mouse model featuring conditional inactivation of the \textit{Pten} tumor suppressor and activation of the \textit{Kras} oncogene targeted to pre-meiotic spermatogonia using Cre/loxP recombination. Interestingly, none of the mice with \textit{Kras} activation alone developed tumors, and only 17\% of mice with \textit{Pten} inactivation alone developed TGCC. However, 75\% of \textit{Pten/Kras} double mutant mice developed TGCC by approximately 4 weeks of age, highlighting a strong synergy between the two oncogenic events. These mice developed teratocarcinoma, a mixed germ cell neoplasm containing both teratoma and embryonal carcinoma components. Consistent with these histopathological features, the \textit{Pten/Kras}-induced TGCCs had scattered clusters of cells that expressed OCT4, a pluripotency promoting transcription factor and marker of human embryonal carcinoma. Metastases containing teratoma tissue and in some cases OCT4-positive cells were identified in 37\% of \textit{Pten/Kras} mice with primary testicular neoplasms. Analysis of the self-renewal and pluripotency properties of the OCT4-positive cells through allograft experiments revealed that TGCCs from \textit{Pten/Kras} mice could be serially transplanted while maintaining a population of OCT4-positive cells. Interestingly, early neoplasms in this model were nearly devoid of the DNA break marker $\gamma$-H2AX, suggestive of distinct DDR properties relative to other solid cancers. Furthermore, preliminary results indicated that cisplatin treatment prolongs the survival of tumor-bearing \textit{Pten/Kras} mice and depletes the OCT4-positive TGCC component. Continued analysis of this model holds great promise for elucidating how stem cell properties of the germ cells from which TGCCs arise impact DDR activity during tumor development and therapy, with important implications for the prevention and treatment of a variety of cancers.
CHARACTERIZATION OF THE DEFECTS IN THE ATP LID OF E. COLI MUTL THAT CAUSE TRANSIENT HYPERMUTABILITY

Pillon, Monica¹, Michelle Dubinsky¹, Randal N. Johnston², Shu-Lin Liu² and Alba Guarne¹
¹Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, L8S 4K1, Canada. ²Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB T2N 4N1, Canada.

The misuse of antibiotics has led to the development of multidrug resistant microbes causing a prevailing hospital epidemic. This natural phenomenon is precipitated by the stress imposed during treatment and can trigger adaptive evolution by increasing the mutation frequency of bacteria in an effort to acquire an advantageous mutation. Inactivation of the DNA mismatch repair (MMR) pathway, whose normal function is to correct replication errors, is often the cause of the mutator phenotype. One of the essential MMR genes, mutL, includes a short tandem repeat prone to polymerase slippage during replication, causing a reversible mutator phenotype. Extensive work has established that this repetitive sequence is a genuine genetic switch; however, the mechanism of MutL inactivation remains unclear. This short tandem repeat is translated into a LALALA motif that resides near the ATPase active site of MutL. We have engineered variants of E. coli MutL with shorter/longer LALALA motifs and characterized their ATPase and DNA binding functions. We have found that the deletion or insertion of a single LA repeat did not compromise the structural integrity of the protein, nor did it affect DNA-binding activity. Changes to the LA repeat also had no effect on forming a complex with MutS. However, they severely compromised ATP binding and the essential ATP-dependent conformational change. Our data suggests that changes to the LA repeat prevent MutL from communicating the presence of a replication error to downstream MMR factors, such as MutH and the UvrD helicase.
Meiosis is the specialized cell division responsible for gamete formation in sexually reproducing organisms. Thus, in order to guarantee the success of a species, it is essential that meiosis is executed correctly. Errors in meiosis can cause infertility or birth defects as a result of aneuploidy, chromosome aberrations, or gene mutations. To prevent these deleterious outcomes, organisms have evolved quality control mechanisms called checkpoints that can eliminate gametes that have irreparable DNA damage or unpaired chromosomes. Our genetic studies have shown that oocytes bearing unrepaired meiotic DSBs are eliminated in a Chk2 dependent pathway. However, there was no strong evidence supporting p53 as the downstream effector of Chk2, as usually observed in mitotic cells. Based on previous studies that indicate high expression of the TAp63 isoform in oocytes, we decided to evaluate its role in meiotic DNA damage response as a potential CHK2 effector. We observed a direct correlation between CHK2 and TAp63 phosphorylation which further supports their mutual role in oocyte death in response to DNA damage. To further analyze this isoform we generated a mouse model lacking TAp63 with persistent unrepaired meiotic DNA breaks in the oocytes. Here, we will present and discuss our results.
Mismatch repair (MMR) corrects DNA polymerase errors that occur during genome replication. MMR is critical for genome maintenance, and its loss increases mutation rates several hundred-fold. Recent work has shown that interaction between the mismatch recognition protein MutS and the replication processivity clamp is important for MMR in several organisms. To further understand how MMR is coupled to DNA replication, we analyzed protein-protein interactions between the mismatch sensing protein, MutS, and the two essential replicases in the bacterium Bacillus subtilis. Strikingly, we found that MutS binds both replicases PolC and DnaE in vitro. Furthermore, using a membrane-permeable crosslinker followed by immunoprecipitating we show that MutS or a MutS allele unable to bind mismatched DNA (MutSF30A), associates with both replicases providing evidence that interactions between MutS and PolC or DnaE occur prior to mismatch detection by MutS. To understand the dynamics of MutS in live cells we performed single molecule imaging of MutS with a photoactivatable fusion (PAmCherry) using PALM. We show that individual MutS-PAmCherry molecules are most often located at the replisome in live cells. While the molecules are at the replisome their movement and speed is restricted. With these data, we propose that MutS directly contacts the DNA replication machinery, and is recruited to nascent DNA allowing MutS to detect mismatches immediately after they are formed.
REGULATION AND KINETICS OF DNA DAMAGE INDUCED PROTEIN RELOCALIZATION IN S. CEREVISIAE

Torres, Nikko P. and Grant W. Brown.
Department of Biochemistry and Donnelly Centre, University of Toronto.

Orchestration of the DNA damage response (DDR) requires an assortment of dynamic post-translational modifications (PTMs), such as phosphorylation, ubiquitination, and sumoylation, to facilitate rapid and adaptive mobilization of repair machinery. Despite the importance of PTMs on the function of the DDR, their influence on the spatial regulation of DDR factors is not well understood. Recently, we systematically characterized DNA damage-induced changes in subcellular localizations of over 4000 GFP-tagged proteins in the model organism, *Saccharomyces cerevisiae*, using high throughput microscopy. From our screen and other studies we have assembled a set of 322 yeast strains expressing GFP-labelled proteins that change in subcellular localization in the presence of DNA damage, and determined the kinetics of their relocalization. We identified 9 and 5 distinct temporal patterns of relocalizations in MMS and HU, respectively. To assess the role of PTMs in regulating the DDR, we are systematically determining the effect of mutating protein kinases, ubiquitin ligases, and SUMO ligases on the relocalization kinetics of these 322 proteins in response to DNA damage. To date we have assessed the effects of deleting the checkpoint kinase gene *RAD53* and of mutating the SUMO E2 *UBC9* on the relocalization dynamics of our protein subset using high throughput microscopy. We found the relocalizations of 62 and 70 proteins in response to MMS-induced DNA damage were altered or abolished in the *rad53Δ* background and *ubc9-10* mutant, respectively. There were 19 proteins that failed to relocalize or had aberrant relocalizations in both mutants, suggesting that phosphorylation and ubiquitination act in concert to regulate the localization of proteins in response to DNA damage. Our study will provide a systematic view of how PTMs regulate the kinetics of DDR protein deployment during DNA damage.
HIGH-THROUGHPUT SCREENING OF DNA2 REGULATORS IN YEAST DNA DAMAGE SIGNALING PATHWAY

Yimit, Askar and Grant W. Brown

Department of Biochemistry and Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, 160 College Street, Toronto, ON M5S 3E1, Canada

DNA damage signaling pathways are crucial for protecting genome stability under different cellular stresses in all eukaryotes. *Saccharomyces cerevisiae* Dna2 has both helicase and nuclease activities that are essential for Okazaki fragment maturation, and is involved in long-range DNA end resection at double-strand breaks. Dna2 forms nuclear foci in response to double-strand breaks and to replication stress. Here we used SGA to cross Dna2-GFP into ~5000 non-essential gene deletion mutants, and determined regulators of Dna2-GFP nuclear focus formation after phleomycin treatment. Dna2-GFP foci were visualized by high throughput confocal microscopy, and 86,000 raw images were analyzed for changes in Dna2-GFP foci. We identified 37 gene deletions that affect Dna2-GFP focus formation, 12 with fewer foci and 25 with increased foci. In addition, we found that Dna2-GFP focus formation occurs mainly in S and G2/M phases, and that the Dna2 N-terminus is important for focus formation in response to replication stress and to double-strand breaks.
Mismatch repair mechanisms improve DNA replication fidelity by reducing the error frequency to 1 in $10^9$ nucleotides incorporated into the nascent DNA strand. In eukaryotes, Msh2-Msh6 (MutS alpha) is responsible for initiating repair of single base mismatches and small insertion/deletion loops, and Msh2-Msh3 (MutS beta) is responsible for repairing larger insertion/deletion loops. Contrary to its positive role in mismatch repair, Msh2-Msh3 is also known to stabilize loops and hairpins formed by repeat nucleotide sequences and thus contribute to triplet nucleotide repeat (TNR) expansion. Our long-term goal is to investigate the mechanism of action of *Saccharomyces cerevisiae* Msh2Msh3 by transient kinetics, in order to understand how it can orchestrate both loop repair and TNR expansion. Toward this end, we are currently testing strategies to express and purify milligram quantities of this large eukaryotic protein complex.

We have successfully expressed large amounts of yMsh2-Msh3 in *E.coli* host cells, and are testing a variety of ion exchange and affinity exchange chromatography methods to optimize its purification. Preliminary biochemical analysis of this recombinant yMsh2-Msh3 complex shows that it binds loop DNA substrates with high affinity. The next step is to analyze the kinetics of yMsh2-Msh3 interactions with different loop and trinucleotide repeat containing substrates, and determine how these interactions are coupled with yMsh2-Msh3 ATPase activity. Our hypothesis is that these kinetic parameters will help distinguish between yMsh2-Msh3 actions in DNA loop repair and TNR expansion.