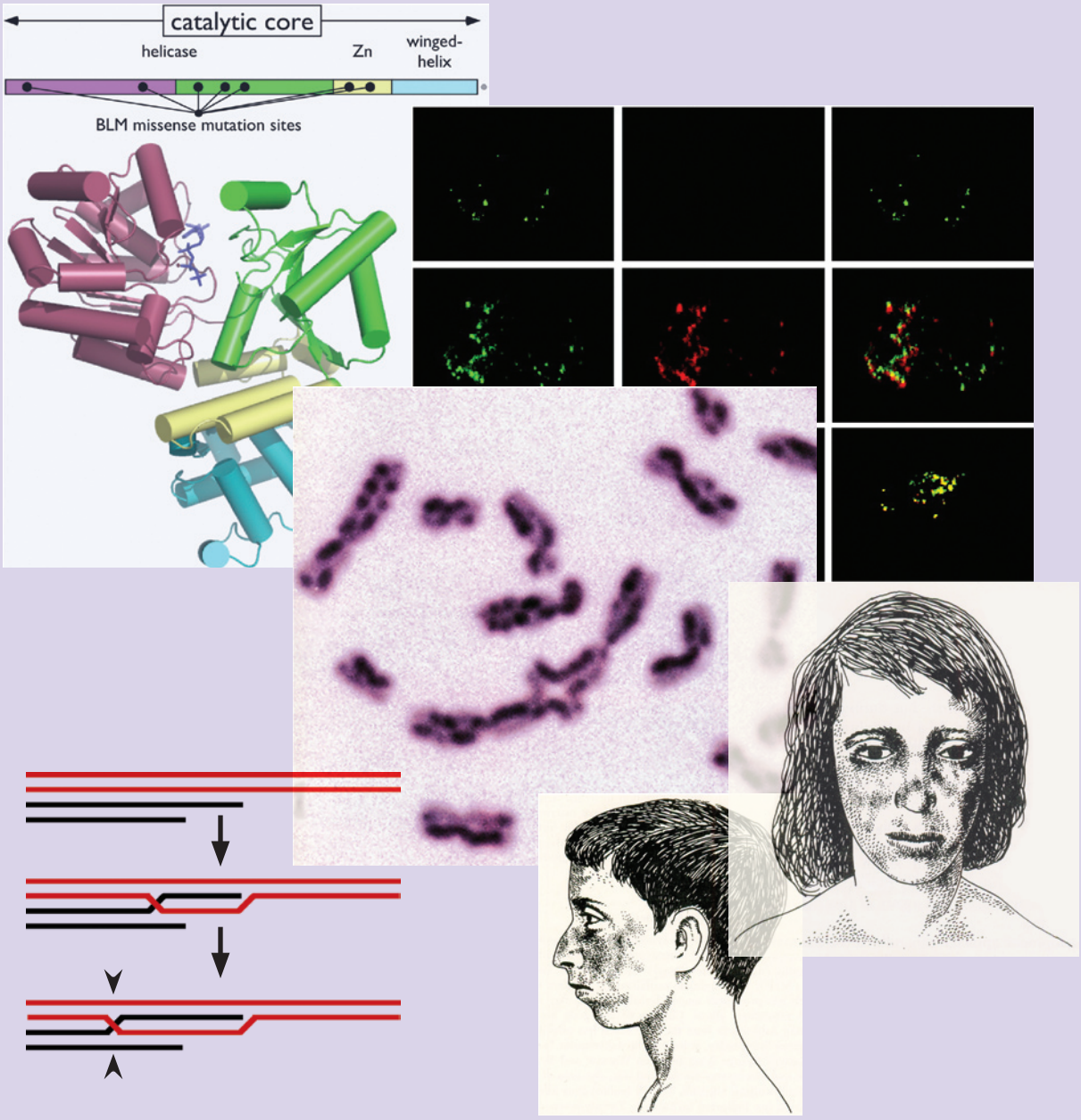
Molecular and Clinical Mechanisms in Bloom's Syndrome and Related Disorders



May 27-28, 2008

University of Chicago Gleacher Center
Chicago, Illinois

THE UNIVERSITY OF CHICAGO
BIOLOGICAL SCIENCES

Molecular and Clinical Mechanisms in Bloom's Syndrome and Related Disorders

Organizers:

Nathan A. Ellis, Vilhelm A. Bohr, and Curtis C. Harris

May 27-28, 2008

University of Chicago Gleacher Center, downtown Chicago, Illinois

CONTENTS

WORKSHOP DESCRIPTION

Background: Bloom's syndrome is a rare autosomal recessive disorder characterized by small size and sun sensitivity. Persons with Bloom's syndrome are also predisposed to the development of many different types of cancer. Bloom's syndrome is caused by mutations in the BLM helicase gene, which is a member of the RecQ family of DNA helicases. The RecQ gene WRN is mutated in Werner's syndrome and RECQL4 is mutated in Rothmund-Thomson syndrome. Both of these syndromes are characterized by premature aging. The RecQ family of helicases is implicated in the interplay between replication stress and repair of DNA damage by homologous recombination and these rare syndromes of the human present models for dissecting the role of these biological processes in the development of cancer and in the processes of aging.

Scientific content: This workshop addresses key research topics surrounding the functions of the RecQ helicases and their influences on cancer and aging. We have brought together leading experts in the field to discuss insights gathered from the study of model genetic organisms and human cells at the intersection between RecQ helicases and the responses to DNA damage. Topics for the meeting include (i) the biochemistry of RecQ helicases, (ii) RecQ helicase function in homologous recombination, (iii) the functions of non-Bloom RecQ helicases, (iv) the roles of RecQ helicases in cancer and aging (v) functions for RecQ helicases in cellular physiology, (vi) mouse and other models of RecQ helicase deficiency, (vii) roles of RecQ helicases in maintenance of genome integrity, and (viii) regulation of recombination by the BLM helicase. We will also discuss the advancement of potential therapies to prevent the genomic instability in Bloom's syndrome.

Scientific goals: The workshop provides opportunities for researchers from a wide spectrum of scientific activities to come together and create new perspectives in understanding the functions of RecQ helicases. Much remains to be learned of the role of BLM in DNA replication, homologous recombination, and DNA repair, and of its function in the maintenance of the genomic integrity. Understanding these functions will help define the mechanisms of genomic instability in Bloom's syndrome cells and elucidate the roles of mechanisms that maintain the integrity of the genome in the development of cancer and in the aging processes.

Format for scientific sessions: Eight one and a half to two hour lecture sessions, two one-hour poster sessions, and a half-hour oral poster presentation overview.

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The Workshop organizers acknowledge the financial support provided by the following governmental institutions and non-governmental organizations and corporations:

Office of Rare Diseases, National Institutes of Health

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National Institute on Aging, National Institutes of Health

The Ellison Medical Foundation

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Bio-Rad Laboratories

Special thanks to the

BLOOM'S SYNDROME FOUNDATION

Program

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DAY 1

8:00 Registration

8:30 **Nathan Ellis** – Welcome

Session 1: *Biochemistry of RecQ helicases*

Session chair: **Vilhelm Bohr**

8:35 **Patrick Sung** – *BLM/Topo IIIα/BLAP-dependent Holliday junction processing*

9:00 **Robert Bambara** – *Protection from illegitimate recombination: BLM helicase function on the lagging strand*

9:25 **James Keck** – *Structural and cellular mechanisms of bacterial RecQ DNA helicases*

9:50 **Poster Presentation Overview**

10:20-10:50 *Break*

Session 2: *RecQ helicase functions in recombination*

Session chair: **Nancy Maizels**

10:50 **Douglas Bishop** – *The energetics of homologous recombination reactions*

11:15 **Alexander Mazin** – *Pro- and anti-recombination activities of the Bloom's syndrome helicase*

11:40 **Yilun Liu** – *RAD51 paralogs and the RecQ helicases – antagonistic roles in homologous recombination?*

LUNCHEON I

12:05-1:00 *Lunch* (6th floor lunchroom)

POSTER SESSION I

1:00-2:00 ***Posters*** (4th floor north lounge)

Session 3: *Functions of the non-Bloom RecQ helicases*

Session chair: **Doug Bishop**

2:00 **Alessandro Vindigni** – *Structural and functional studies on the human RECQ1 helicase*

2:25 **Pavel Janscak** – *Biochemistry and function of RECQL5 protein*

2:50 **Igor Stagljar** – *Acetylation of RECQL4, the Rothmund-Thomson-,RAPADILINO- and Baller-Gerold-Syndrome gene product, by the histone acetyltransferase p300 regulates its subcellular localization*

3:15-3:45 *Break*

Session 4: *ELLISON MEDICAL FOUNDATION SESSION: RecQ functions in cancer and aging*

Session chair: **Nathan Ellis**

3:45 **Vilhelm Bohr** – *Human RecQ helicase function in double- and single-strand DNA repair*

4:10 **Patricia Opresko** – *RecQ helicases' functions at telomeres*

4:35 **Ray Monnat** – *The Werner syndrome protein WRN as a fork 'spork'*

5:00 - 5:30 *Break*

EVENING RECEPTION (with **Bloom's Connect**– 6th floor Board room)

Session chairs: **Nathan Ellis and Vilhelm Bohr**

5:30 - 6:00 *Reception*

6:00 **James German** – *Clinical investigation and basic research: Bloom's syndrome and discovery*

6:15 **Maureen Sanz** – *Report from the Bloom's Syndrome Registry*

6:30 **Richard Gladstein** – *Mission of the Bloom's Syndrome Foundation*

DAY 2

Session 5: *RecQ helicases and cellular physiology*

Session chair: **Joanna Groden**

8:30 **Nancy Maizels** – *RecQ helicases and the maintenance and expression of G-rich human genes*

8:55 **Robert Brosh** – *Mechanisms of RecQ-like helicases in cellular DNA metabolism*

9:20 **Yves Pommier** – *BLM and replication stress responses: single-cell, single-DNA molecule and pharmacological analyses*

9:45-10:15 *Break*

Session 6: *Models of RecQ helicase deficiency*

Session chair: **Curtis Harris**

10:15 **Joanna Groden** – *Tissue-specific effects of Blm haploinsufficiency on murine tumor initiation, progression and regression*

10:40 **Guangbin Luo** – *Interrogating the molecular mechanisms of RecQ helicase functions using knockout models*

11:05 **Shunichi Takeda** – *The reverse genetic study of the vertebrate Blm and Fbh1 DNA helicases using the chicken DT40 cell line and Medaka fish*

11:30 **Jeff Sekelsky** – *Analysis of synthetic lethality phenotypes provides insights into functions of Drosophila BLM in maintaining genome stability*

LUNCHEON II

11:55-1:00 *Lunch* (6th floor lunchroom)

POSTER SESSION II

1:00-2:00 ***Posters*** (4th floor north lounge)

Session 7: *RecQ helicases in genome integrity*

Session chair: **Vilhelm Bohr**

2:00 **Steve Brill** – *Role of SUMO in the absence of the yeast Sgs1 DNA helicase*

2:25 **Qin Yang** – *RecQ helicases, telomere recombination and maintenance*

2:50 **Weidong Wang** – *A multiprotein complex that maintains genome stability and is involved in Bloom syndrome and Fanconi Anemia*

3:15-3:40 *Break*

Session 8: *Regulation of recombination by the BLM helicase*

Session chair: **Ray Monnat**

3:40 **Nathan Ellis** – *SUMO modification of BLM and the regulation of anti-recombination*

4:05 **Hocine Mankouri** – *S. cerevisiae sgs1 mutants as a model system to develop strategies to suppress Bloom's syndrome (BS) phenotypes*

4:30 **Ralph Scully** – *Control of sister-chromatid recombination in mammalian cells*

4:55 **Nathan Ellis and Vilhelm Bohr** – *Final comments*

5:00 *Meeting ends*

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SPEAKER ABSTRACTS

DAY1 – Session 1: Biochemistry of RecQ helicases

BLM/Topo IIIα/BLAP-dependent Holliday junction processing

Steven Raynard[1], Wendy Bussen[1], Wilson Zhao[1], Valeria Busygina[1], Amom Ruhikanta Meetei[2], and Patrick Sung[1]

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BLM helicase, Topo IIIα, BLAP75 combine to form an evolutionarily conserved complex, termed the BTB complex, that functions to regulate homologous recombination (HR) by branch migrating the Holliday junction (HJ) and dissolving the double Holliday junction (dHJ) to yield non-crossover recombinants. These attributes of the BTB complex are reliant on the ability of BLM to hydrolyze ATP. BLAP75 binds DNA and interacts directly with both BLM and Topo IIIα. With the use of a series of BLAP75 protein fragments, we show that the evolutionarily conserved amino-terminal third of BLAP75 mediates complex formation with BLM and Topo IIIα and that the DNA binding activity resides in the carboxy-terminal third of this novel protein. Interestingly, the amino terminal third of BLAP75 is just as adept as the full-length protein in the promotion of dHJ dissolution and HJ unwinding by BLM-Topo IIIα. Thus, the BLAP75 DNA binding activity is dispensable for BTB complex's ability to process the HJ *in vitro*. Lastly, we show that a BLAP75 point mutant, defective in Topo IIIα interaction, is unable to promote dHJ dissolution and HJ unwinding by BLM-Topo IIIα. This result speaks to the functional significance of specific protein-protein interactions within the BTB complex.

Protection from illegitimate recombination: BLM helicase function on the lagging strand

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DNA replication in human cells involves synthesis and joining of millions of RNA-initiated Okazaki fragments. One pathway for RNA removal is the creation of single stranded flaps 10-50 nucleotides long, containing the initiator RNA segments, and then removal of the flaps before fragment joining. When they grow to more than 15 nucleotides, flaps can interact with the single-stranded binding protein RPA. This protein delays access by flap endonuclease-1 (FEN1), the specialized nuclease needed for flap removal, increasing the flap lifetime. RPA has a strand melting property that assists polymerases and helicases in resolving DNA secondary structures. This initially suggested to us that the RPA coating would protect the flap from participating in illegitimate recombination contributing to genome instability. However, in a model system for recombination, we found that RPA-coated flaps could readily bind and ligate at a complementary ectopic site to initiate recombination.

We later found that many natural complementary single strands anneal poorly because of stable intra-molecular hydrogen bonding. RPA transiently melts the single strands, so that they can bind to form double strands. In this way melting ironically promotes annealing. Structured single strands achieve an equilibrium with double strands, a consequence of RPA driving both annealing and melting. Results suggest that displaced flaps with secondary structure formed during Okazaki fragment maturation can be melted by RPA and subsequently annealed to a complementary ectopic DNA site to initiate recombination.

We found that Bloom Protein (BLM) helicase activity, exhibited in the presence of ATP, can directly disrupt annealing at the ectopic site and promote FEN1 cleavage. BLM has its own strand annealing and strand exchange activities. RPA inhibited the BLM strand annealing activity, thereby promoting helicase activity and complex dissolution. In this sense the presence of RPA and BLM together supports the anti-recombination function of BLM helicase. BLM also can employ strand exchange to readily dissociate invading flaps, e.g. in a D-loop, if the exchange step does not involve annealing of RPA-coated strands. Use of ATP to activate the helicase function did not aid flap displacement by exchange, suggesting that this is a helicase-independent mechanism of complex dissociation.

In summary, RPA coated flaps, intermediates of normal DNA replication, can participate in recombination that disrupts genome integrity. However, BLM interacts with RPA to exhibit helicase function that prevents annealing to initiate recombination. Moreover, BLM can disrupt D-loop recombination intermediates by an exchange function that is independent of helicase activity and RPA.

Structural and cellular mechanisms of bacterial RecQ DNA helicases

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RecQ family helicases play important roles in coordinating genome maintenance pathways in cells. In the absence of functional RecQ proteins, cells exhibit a variety of phenotypes, including increased mitotic recombination, elevated chromosome missegregation, hypersensitivity to DNA damaging agents, and defects in meiosis. Mutations in three of the five human RecQ genes give rise to genetic disorders associated with a predisposition to cancer and premature aging, highlighting the importance of RecQ proteins and their cellular activities for human health. Current evidence suggests that RecQ proteins act at multiple steps in DNA replication, including stabilization of replication forks and removal of DNA recombination intermediates. I will describe our latest structural and biochemical work on RecQ proteins from *Escherichia coli*. *E. coli* RecQ was the first identified RecQ family member and contains the major domains that are conserved in nearly all RecQs. We have determined the high-resolution structures of these domains (catalytic core and Helicase-and-RNaseD C-terminal (HRDC) domains). In addition, we have identified proteins that associate with *E. coli* RecQ *in vivo*; the identity of these proteins helps explain the cellular roles of RecQ proteins in a simple bacterial model system.

Our RecQ studies are supported by a grant from the NIH (GM068061).

Selected references:

Killoran MP, Keck JL. Sit down, relax, and unwind: structural and functional mechanisms of RecQ DNA helicases. **Nucl Acids Res** 2006; *34*:4098-4105

Shereda RD, Bernstein DA, Keck JL. A central role for SSB in *E. coli* RecQ DNA helicase function. **J Biol Chem** 2007; *282*:19247-19258

Killoran MP, Keck JL. Structure and function of the regulatory C-terminal HRDC domain from *Deinococcus radiodurans* RecQ. **Nucl Acids Res** 2008 (in press)

Poster Presentation Overview

1. **Robert Shereda** – *SSB: Organizational center and Achilles heel*
2. **Natalie C. Fonville** – *The death-by-recombination pathway promoted by E. coli RecQ*
3. **Steven Raynard** – *Functional role of BLAP75 in BLM/Topo IIIα-dependent Holliday junction processing*
4. **Rong Guo** – *BLAP15/RMI2 is a new component of the Bloom syndrome complex and essential for maintaining genome integrity*
5. **Yuliang Wu** – *FANCJ helicase defective in Fanconia anemia and breast cancer unwinds G-quadruplex DNA to defend genomic stability*
6. **Gregory Sowd** – *Mechanism and substrate specificity of telomeric protein POT1 stimulation of the Werner syndrome helicase*
7. **Daniela Huehn** – *Functional characterisation of the human RECQL5 helicase*
8. **Lisa Wang** – *Response of Rothmund-Thomson syndrome fibroblasts to genotoxic agents*
9. **Beatriz Russell** – *Interaction of the BLM helicase and topoisomerase IIα modulates helicase activity and protects against chromosome breaks*
10. **Jeremy Keirsey** – *Novel phosphorylation sites in BLM regulate localization to stalled replication forks and helicase activity*
11. **Sabrina Andersen** – *Characterizing the genetic requirements of hyper-recombination in Drosophila BLM mutants*
12. **Wai-kit Chu** – *Genetic analysis of homologous recombination repair pathways in mouse embryonic stem cells*
13. **Jeannine LaRocque** – *Mechanisms of gene conversion in wild-type and Msh2 mutant mammalian cells*
14. **Andrea Hartlerode** – *Distinct roles of chromatin-associated factors MDC1 and 53BP1 in mammalian double-strand break repair*
15. **Jason Aulds** – *RecQ helicases and their role in oxidative DNA damage repair at the telomere*
16. **Birija S. Patro** – *A collaborative role of Werner syndrome protein and ATR in replication stress induced double-strand break repair*
17. **Gerald Nora** – *Multiplex analysis of TRF2 modulation of WRN helicase/exonuclease dissociation of HJ DNA*
18. **Patrick Grierson** – *Functional analysis of the BLM helicase in RNA polymerase-I transcription and characterization of its helicase activity on RNA:DNA substrates*

The energetics of homologous recombination reactions

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Strand exchange recombinase proteins of the RecA family bind to both double and single strand DNA with high affinity. Energy provided by ATP hydrolysis is required for recombinase proteins to dissociate from both types of DNA. Although this expenditure of energy may serve to drive recombination reactions forward by dissasembling recombinase filaments after strand exchange, it has become clear that energy-dependent removal of filaments occurs in two different non-recombinogenic contexts. Removal of strand exchange proteins from ssDNA has been shown to remove recombinases from ssDNA thereby preventing un-necessary and or detrimental recombination events such as those that characterize Bloom syndrome. In addition, both prokaryotic and eukaryotic cells expend energy to dissociate recombinase from dead-end complexes on dsDNA. We will present data showing that this process occurs in both pro- and eukaryotes, albeit by very different mechanisms. Given these findings, its reasonable to ask why haven't recombinases evolved to avoid formation of non-functional complexes on dsDNA? We propose that the need to expend energy in this way is a direct consequence of the mechanism of the strand exchange reaction itself.

We will present data showing that dead-end complexes form as a consequence of disrupting recombination associated ATP hydrolysis in both yeast and bacteria. Some of this data involves the use of novel method for characterizing the structure of bacterial chromosomes. We will also present a model in which a critical function of energy in recombination reactions is the disassembly of recombinase filaments from strand exchange products and from sites on dsDNA where non-productive assembly of recombinase has formed structures that resemble these products.

Pro- and anti-recombination activities of the Bloom's syndrome helicase

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Mutations in *BLM* helicase, a member of the conserved RecQ family, cause an autosomal disorder called Bloom's syndrome (BS). The hallmark of BS is a high degree of genome instability, which is responsible for frequent incidence of cancer. BLM helicase plays a role in homologous recombination, however its exact function remains controversial. Mutations in *BLM* cause hyper-recombination between sister chromatids and homologous chromosomes, indicating an anti-recombination role. Conversely, other data show that BLM is required for recombination. It was previously shown that *in vitro* BLM helicase promotes disruption of recombination intermediates, regression of stalled replication forks, and dissolution of double Holliday junctions. These activities were initially attributed to an anti-recombination function of BLM. However, recent findings in our and other laboratories indicate a broader role of BLM: BLM can both promote and inhibit homologous recombination at different stages, thereby imposing an effective control over homologous recombination[1]. We found two novel activities of BLM: disruption of the RAD51-ssDNA filament, an active species that promotes homologous recombination, and stimulation of DNA repair synthesis by DNA polymerase η. We also found that the disruption of the filament by BLM only occurs when RAD51 is present in an inactive ADP-bound form. Our new results on BLM interactions with the RAD51 filament demonstrated remarkable similarities in the biochemical properties of BLM and RAD54 proteins. These findings reveal new possible roles of BLM in homologous recombination and show a profound effect of the RAD51 conformation on the function of BLM in homologous recombination.

1. Bugreev DV, Yu X, Egelman EH, Mazin AV. Novel pro- and anti-recombination activities of the Bloom's syndrome helicase. **Genes Dev** 2007; *21*:3085-3094

RAD51 paralogs and the RecQ helicases – antagonistic roles in homologous recombination?

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The integrity of our chromosomal material is dependent upon the efficient repair of DNA lesions that are caused by exogenous agents such as ionizing radiation, ultraviolet radiation or interstrand crosslink agents. In mammalian cells, multiple DNA repair mechanisms and machineries have evolved to remove a particular type of DNA lesion. The decision of which repair machinery to implement depends on how the lesion is detected and during what cellular process or which cell-cycle stage the lesion is introduced. A tight communication, collaboration and even competition among different DNA repair pathways are essential for efficient removal of DNA damage and restoration of genome integrity. Without such comprehensive DNA repair and surveillance systems, broken chromosomes and mutations accumulate in cells leading to apoptosis, which could have devastating consequences in growth and development of an organism. Two families of DNA repair proteins, the RAD51 paralogs and the RECQ family helicases, are examples of tumor suppressors that participate in multiple and often antagonistic pathways to restore the integrity of our genome. In mammals, each of the RAD51 paralog and RECQ families consists of five homologs. The five RAD51 paralogs are RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3. Genetic analyses indicate that each of the RAD51 paralogs plays critical and non-redundant role in DNA repair. Similarly, there are five RECQ homologs in human, and they are RECQ1, BLM, WRN, RECQ4 and RECQ5. Mutations in different RECQ helicases have been associated with distinct clinical diseases and genome instability phenotypes. Our work on dissecting protein-protein interactions and biochemical activities associated with different RAD51 paralogs and the RECQ helicases provide us insight into the functions of these proteins and their roles as genome caretakers.

Structural and functional studies on the human RECQ1 helicase

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RecQ helicases are a ubiquitous family of DNA strand separating enzymes that defend the genome against instability. Our recent findings highlight several differences between the substrate specificities of RECQ1 and BLM, providing a strong indication that these helicases are likely to perform non-overlapping functions in cells. In particular, RECQ1 cannot unwind G-quadruplexes or RNA:DNA hybrid structures. Moreover, RECQ1 cannot substitute for BLM in the regression of a model replication fork and is not able to displace plasmid D-loops lacking a 3' tail. Conversely, RECQ1, but not BLM, is able to resolve immobile Holliday junction structures lacking a homologous core, even in the absence of hRPA. We have previously demonstrated that two different oligomeric structures, higher-order oligomers consistent with pentamers or hexamers, and smaller oligomers consistent with monomers or dimers are associated with the strand annealing and unwinding activity of RECQ1, respectively[1]. Our mutagenesis studies show that the N-terminal region (1-56) of RECQ1 is necessary for higher-assembly state formation and confirm that smaller oligomers are involved in DNA unwinding. The N-terminal region is however necessary for the Holliday junction resolution activity of RECQ1 suggesting that this domain, or the higher-assembly state formation promoted by the N-terminus, is essential for the ability of the protein to resolve Holliday junctions. A truncated form of RECQ1 lacking the first 48 residues and the last 33 amino acids at the C-terminus ($RECQL^{49-616}$) was crystallized in presence of $MgCl_2$ and ATPγS with a resolution of 2.0 Å. The crystal structure of RECQ1 shows that the relative position and orientation of the zinc-binding motif and the Winged Helix (WH) that form the RQC domain of RECQ1, is different from that of *E.coli* RecQ (pdb code: 2V1X). Moreover, the structure shows a prominent beta hairpin, with a tyrosine residue at the tip, located in the wing of the WH domain of RECQ1 that is much shorter in the structures of the WH domains of *E.coli* RecQ and WRN. Our mutagenesis experiments indicate that this hairpin plays a crucial role in DNA strand separation, as already suggested for other helicases of the SF2 family, and may represent an important determinant for the distinct substrate specificity of the five human RecQ enzymes.

1. Muzzolini L, Beuron F, Patwardhan A, Popuri V, Cui S, Niccolini B, Rappas M, Freemont PS, Vindigni, A. Different quaternary structures of human RECQ1 are associated with its dual enzymatic activity. **PLoS Biol** 2007; *5*, e20.

Biochemistry of RECQL5 protein

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The mammalian RECQL5 protein is a tumor suppressor that plays a role in the control of homologous recombination. The goal of our research is to elucidate the exact DNA transactions mediated by RECQL5 *in vivo*. Our biochemical studies with recombinant human RECQL5 protein indicated that RECQL5 functions as an ATP-dependent 3'-5' DNA helicase with the ability to promote migration of Holliday junctions. However, unlike other RecQ helicases, RECQL5 required a single-stranded DNA binding protein to mediate DNA unwinding. Surprisingly, we found that RECQL5 possesses a strong DNA strand-annealing activity residing in the unique C-terminal region of the protein. The strand-annealing activity of RECQL5 was completely abolished by the binding of ATPgS to the helicase domain of the enzyme, suggesting that the helicase and strand-annealing domains of RECQL5 act in a concerted fashion. In agreement with this hypothesis, RECQL5 was found to efficiently promote strand exchange on forked DNA structures with homologous arms to yield four-way junctions.

Our cellular studies using immunofluorescence microscopy showed that RECQL5 localized to DNA replication factories and persisted at the sites of stalled DNA replication forks. We also found that RECQL5 was rapidly recruited to laser-induced DNA double-strand breaks (DSBs) where it persisted even an hour following irradiation. Mass spectrometric analysis of RECQL5 immunoprecipitates from human cell extracts revealed that RECQL5 interacts with the MRE11/RAD50/NBS1 (MRN) complex that plays a central role in the signaling and recombinational repair of DSBs. Pull-down experiments with purified recombinant proteins suggested that the formation of the RECQL5/MRN complex is mediated by direct binding of RECQL5 to MRE11 and NBS1. Functional assays revealed that RECQL5 inhibited the 3'-5' exonuclease activity residing in the MRE11 protein. At the cellular level, the MRE11 protein was found to be required for the recruitment of RECQL5 to sites of arrested replication forks and sites of laser-induced DSBs. Collectively, our data suggest a functional relationship between RECQL5 and the MRN complex in the cellular response to DNA damage.

Acetylation of RECQL4, the Rothmund-Thomson-,RAPADILINO- and Baller-Gerold-Syndrome gene product, by the histone acetyltransferase p300 regulates its subcellular localization

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RECQL4 is a member of the conserved RecQ family of DNA helicases that play important roles in the maintenance of genome stability in all organisms examined. Although it was reported that genetic alterations in the RECQL4 gene are associated with three autosomal recessive disorders called Rothmund-Thomson, RAPADILINO, and Baller-Gerold syndromes, respectively, the molecular role of RECQL4 still remains poorly understood. Here we show that RECQL4 specifically interacts with the histone acetyltransferase p300 both, *in vivo* and *in vitro*, and that p300 acetylates one or more of the lysine residues at positions 376, 380, 382, 385, and 386 of RECQL4. Furthermore, we report that these five lysine residues lie in a short motif of 30 amino acids that is essential for the nuclear localization of RECQL4. Remarkably, the acetylation of RECQL4 by p300 *in vivo* leads to a significant shift of a proportion of RECQL4 protein from the nucleus to the cytoplasm. This accumulation of the acetylated RECQL4 is due to its inability to get imported into the nucleus. Our results provide first evidence of a post-translational modification of the RECQL4 protein and suggest that acetylation of RECQL4 by p300 regulates the trafficking of RECQL4 between the nucleus and the cytoplasm.

Human RecQ helicases function in double and single strand DNA repair

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The 5 human RecQ helicases protect the genome against genomic instability and participate in many DNA metabolic processes. Three of them, Werner syndrome (WRN), Bloom syndrome (BLM) and Rothmund Thomson (RTS), are deficient, each in a specific syndrome associated with premature aging and increased malignancies. These RecQ helicases appear to operate at stalled replication forks, and to be involved in DNA repair.

Studies from ours and other laboratories have suggested that WRN is involved in double- and single stranded DNA repair, and we are investigating whether this also is the case for BLM and RTS.

Recent results from these studies will be discussed, including biochemical and cellular studies of the function of RTS that implicate this protein in double- and single stranded DNA repair pathways.

RecQ helicases functions at telomeres

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We are investigating roles for RecQ helicases in DNA replication and repair at telomeres. Defects in the RecQ helicases WRN or BLM result in cancer-prone and premature aging disorders in humans; Werner Syndrome (WS) and Bloom Syndrome (BS), respectively. Cellular phenotypes of WS and BS, including genomic instability and premature senescence, are consistent with telomere dysfunction. WRN and BLM are proposed to function in dissociating alternate DNA structures during DNA replication and recombination pathways at telomeric ends.We found previously that the telomeric single strand binding protein POT1 strongly stimulates WRN and BLM helicases to unwind telomeric fork and D-loop structures. However, POT1 does not stimulate *E. coli* RecQ helicase. We are continuing to investigate the cellular specificity of the POT1 physical interaction with WRN protein and the mechanism for the helicase stimulation. We reasoned that POT1 stimulation of WRN helicase could result from POT1 recruitment and retention of WRN on telomeric substrates and/or POT1 loading on partially unwound substrates to prevent strand reannealing. POT1 pre-loading inhibits WRN activity on a telomeric duplex with a 3' telomeric tail, but stimulates WRN helicase on a telomeric forked duplex with a 3' telomeric tail and an additional 5' tail.In contrast, RPA stimulates WRN activity on both substrates. This is consistent with a role for POT1 in preventing fraying of an open telomeric end, but also in facilitating processing of replication or recombination type forked structures at the telomeres. We next constructed plasmid-based telomeric D-loop structures so that the telomeric sequence is present both in the duplex region (84 base pairs) of the invading strand, and the displaced ssDNA region. WRN helicase releases a mixture of full length invading strands and shortened strands due to the action of the exonculease. The addition of POT1 did not alter the percent of D-loops unwound by the WRN helicase, but did increase the percent of intact strands released and limited WRN exonuclease. Collectively, these data suggest POT1 stimulates WRN helicase by maintaining partially unwound strands in a melted state, rather than serving as a processivity clamp for retaining WRN on the substrate. These findings support a role for the cooperation of POT1 with WRN, and potentially BLM, in resolving telomeric DNA structures for replication and/or recombination.

The Werner syndrome protein WRN as a fork 'spork'

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Several *in vivo* functions have been attributed to the Werner syndrome protein WRN in different aspects of DNA metabolism. These functions include roles in recombination, replication and repair, and in telomere maintenance and transcription. Reconciling this diversity of roles has spawned models for *in vivo* function in which the WRN exonucle-ase and helicase activities act alone, or in conjunction with additional proteins, on DNA junctions or partial duplex DNA molecules.

These models envision the WRN protein as a biochemical equivalent of the 'spork', that delightful culinary hybrid that combines the useful properties of a spoon and fork in a single implement that can be used to perform a wide range of activities on many different culinary substrates. The spork will serve as our metaphor for exploring different roles for the human WRN protein in replication, recombination and genome maintenance.

We will present recent data on the role of WRN in replication fork progression after DNA damage (base adduct damage or replication fork arrest with hydroxyurea) that does not postulate or require a direct role in replication fork remodeling or stabilization 1. These results will be discussed in light of recent data that suggest a complementary role for the human Bloom syndrome protein BLM in replication fork regulation after DNA damage. The most interesting implication of these two stories is that the human WRN and BLM syndrome proteins may perform multiple, partially redundant and complement-tary functions in DNA replication and recombination to ensure genome replication and genomic stability.

Experimental tests that could be used to explore this model will be discussed, as will implications for human disease risk and the response to therapy.

This work is supported by US National Institutes of Health awards to RM (CA77852), JS (a CA103720 sub-award) and AF (R21/R33 EB003307).

1. Sidorova JM, Li N, Folch A, Monnat RJ Jr. The Werner syndrome RecQ helicase is required for normal replication fork progression after DNA damage or replication arrest. **Cell Cycle** 2008 (in press)

Bloom's syndrome at fifty

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Persons with Bloom's syndrome (BS) and their families have participated in a nearing-five-decades-long program that is referred to as the Bloom's Syndrome Registry. The program has defined the clinical syndrome itself, and, importantly, it has shown that persons with BS are greatly predisposed to several major diseases, the most frequent being chronic obstructive lung disease, diabetes mellitus, and malignant neoplasia.

The rationale behind the surveillance of the BS population, itself very rare – witness fewer than 300 persons having become known to us, to be accessioned to the Registry – is that understanding the etiology and pathogenesis of these common disorders (BS's clinical complications just mentioned) will be applicable in the general population. The information gathered also not infrequently has direct benefits to the participating persons themselves.

Data accumulated in the Registry to be reported will include the following, all in comparison to the general population: vital statistics including the age distribution of the registered population; nationalities, religions, and ethnic groups; incidence of complications; fertility status; the accumulation and cryopreservation of biological specimens including cell lines. In addition, these various parameters will be examined in affected individuals (with BS) in different classes of causative mutations (frameshift and nonsense mutations, missense mutations, splicing defects).

RecQ helicases and the maintenance and expression of G-rich human genes

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BLM helicase and other RecQ family helicases carry an unusual, conserved domain, the RQC domain. The RQC domain promotes high affinity interactions with G4 DNA. G4 DNA (also known as G-quadruplex DNA or G-tetraplex DNA) is a four-stranded structure formed by G-rich sequences, in which intra- or inter-strand interactions are stabilized by G-quartets, planar arrays of four guanines, paired by Hoogsteen bonding. G4 DNA forms spontaneously in regions which contain at least four runs of guanines, with at least three guanines per run. RNA can form a similar structure, and G4 DNA and G4 RNA are both very stable once formed.

To identify human genes which are dependent upon BLM and other RecQ family members for their maintenance and expression, we quantitated the potential for G4 DNA formation ("G4P") of all human genes, and then correlated gene function with G4P. This revealed that very low and very high G4P correlates with specific functional classes of genes. Tumor suppressor genes have very low G4P and proto-oncogenes have very high G4P. These results show that genomic structure undergoes selection based on gene function. G-rich regions are unstable, so selection based on G4P could promote genomic stability (or instability) of specific classes of genes. Selection based on G4P could also reflect mechanisms for global regulation of gene expression.

To understand how potential for G-quadruplex formation might influence regulation of gene expression, we examined the 2 kb spanning the transcription start sites (TSS) of all human genes, distinguishing contributions of template and nontemplate strands. Regions both upstream and downstream of the TSS are G-rich, but the downstream region displays a clear bias toward G-richness on the nontemplate strand. Upstream of the TSS, much of the G-richness and G4P derives from the presence of well-defined canonical regulatory motifs in duplex DNA, including CpG dinucleotides which are sites of regulatory methylation, and motifs recognized by the transcription factor SP1. Downstream of the TSS, G-richness is concentrated in the first intron, and on the nontemplate strand, where polymorphic sequence elements with potential to form G-quadruplex structures, and which cannot be accounted for by known regulatory motifs, are found in almost 3000 (16%) of the human RefSeq genes, and are conserved through frogs. These elements could in principle be recognized either as DNA or as RNA, providing structural targets for regulation of gene expression.

Mechanism of RecQ-like helicases in cellular DNA metabolism

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Helicases are molecular motor proteins that couple ATP hydrolysis to nucleic acid unwinding. The growing number of DNA helicases implicated in human disease suggests that their vital specialized roles in cellular pathways are important for the maintenance of genome stability. Of particular interest is the RecQ-like family of DNA helicases that play important roles in the DNA damage response. Understanding the cellular mechanisms whereby RecQ-like helicases preserve chromosomal integrity is projected to help us understand the fundamental basis of age-related disease and cancer. This topic will be discussed with an emphasis on our latest development of model systems to study the functions of RecQ-like helicases.

BLM and replication stress responses: single-cell, single-DNA molecule and pharmacological analyses

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We will present studies showing that cells derived from Bloom's Syndrome patients accumulate replication-associated DNA double-strand breaks (Rep-DSB) during normal cell cycle. Such Rep-DSB can be detected as histone g-H2AX foci that colocalize with replication foci and with the DNA damage response (DDR) proteins: phosphorylated ATM on serine 1981 (S1981-P-ATM) and phosphorylated Chk2 on threonine 68 (T68-P-Chk2)1. Single-DNA molecule analyses by "molecular combing"[2] showed that BLM-deficient cells exhibit replication fork breakage, reduced average fork velocity and increase origin frequency, which are all indicative of global defects in fork progression with compensatory reactivation of cryptic replication origins in BLM-deficient cells[1].

We will also present studies demonstrating the involvement of BLM in DNA replication stress response elicited by replication inhibitors. Camptothecin (CPT) is a selective topoisomerase I inhibitor, well-known for inducing Rep-DSB when a replication fork encounters a Topoisomerase I-DNA cleavage complex[3]. CPT induces the rapid phosphorylation of BLM on serine T99 (T99-P-BLM)[4]. We have developed specific antibodies for T99-BLM and shown that T99-BLM formation in response to CPT is ATM-dependent[4]. T99-BLM forms nuclear foci that colocalize with S1981-P-ATM, T68-P-Chk2 and g-H2AX indicating that T99-BLM formation is coordinated with the DDR pathways elicited by Rep-DSB. Accordingly, BLM-deficient cells are deficient for g-H2AX activation[4] and are hypersensitive to CPT[4]. We will also present recent evidence[5] that BLM helicase, ATR kinase, and Mus81 nuclease are required to convert perturbed replication forks to DNA breaks when cells encounter conditions that decelerate DNA replication, thereby leading to the rapid repair of those breaks and resumption of DNA replication without incurring DNA damage and without activating a cell cycle checkpoint.

1. Rao VA, et al. Endogenous γH2AX-ATM-Chk2 checkpoint activation in Bloom's syndrome helicase deficient cells is related to DNA replication arrested forks. **Mol Cancer Res** 2007; *5*:713-724

2. Conti C, et al. Replication fork velocities at adjacent replication origins are coordinately modified during DNA replication in human cells. **Mol Biol Cell** 2007; *18*:3059-3067

3. Pommier Y. Topoisomerase I inhibitors: camptothecins and beyond. **Nat Rev Cancer** 2006; *6*:789-802

4. Rao A, et al. Phosphorylation of BLM, dissociation for topoisomerase IIIa and colocalization with γH2AX after topoisomerase I-induced replication damage. **Mol Cell Biol** 2005; *25*:8925-8937

5. Shimura T, et al. Bloom's syndrome helicase and Mus81 are required to induce transient double-strand DNA breaks in response to DNA replication stress. **J Mol Biol** 2008; *375*:1152-1164

Tissue-specific effects of *Blm* haploinsufficiency on murine tumor initiation, progression and regression

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Examination of The Bloom's Syndrome registry suggests that the strong cancer predisposition in persons with BS does not affect all organ systems equally[1,2]. For example, adult persons with BS have a significant risk for colorectal cancer[3], but are not affected as often by other tumor types. Heterozygosity for BLM mutation is also associated with an increased risk for colon cancer in one study[3] but not breast cancer[4] or colon cancer in two other studies[5,6]. To evaluate the effects of BLM mutation on tumor formation, we examined two mouse models of human cancer that are characterized by adenoma formation in the lung and intestine. We tested whether haplosufficiency of Blm would alter the number, size and histopathology of adenomas in a murine model of intestinal tumor formation and in an inducible lung tumor model. Blm haploinsufficiency led to intestinal adenomas with high grade dysplasia and carcinoma in ApcMin/+ mice, but had no impact on tumor development, progression or regression in mice with lung-specific overexpression of Fgf-10. This suggests potentially different roles for the BLM helicase in lung and intestinal epithelium, and also suggests that genes and alleles may modify tumor susceptibility differently in various tissues.

1. German J. Bloom's syndrome. XX. The first 100 cancers. **Cancer Genet Cytogenet** 1997; *93*:100-106

2. Lowy AM, Kordich JJ, Gismondi V, Varesco L, Blough RI, Groden J. Numerous colonic adenomas in an individual with Bloom's syndrome. **Gastroenterology** 2001; *121*:435-439

3. Gruber SB, et al. BLM heterozygosity and the risk of colorectal cancer. **Science** 2002; *297*:2013

4. King MC, Marks JH, Mandell JB, New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. **Science** 2003; *302*:643-646

5. Cleary SP, Zhang W, Di Nicola N, Aronson M, Aube J, Steinman A, Haddad R, Redston M, Gallinger S, Narod SA, Gryfe R. Heterozygosity for the BLM(Ash) mutation and cancer risk. **Cancer Res** 2003; *63*:1769-1771

6. Zauber NP, Sabbath-Solitare M, Marotta S, Zauber AG, Foulkes W, Chan M, Turner F, Bishop DT. Clinical and genetic findings in an Ashkenazi Jewish population with colorectal neoplasms. **Cancer** 2005; *104*:719-729

Interrogating the molecular mechanisms of RecQ helicase functions using knockout models

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We have been using knockout mouse animal models and knockout cell models to study the roles of individual member of the *RecQ* family in cancer suppression, and to interrogate the molecular mechanisms by which these helicases maintain genome integrity. To this end, we have generated and completed the characterization of *Recql4-, Blm-,* and *Recql5*-knockout mice. The results from these studies showed that all these three types of knockout mice are predisposed to cancer, and they are cancer prone because of three distinct underlying mechanisms of genome instability. Recql4 knockout resulted in a unique premature centromere separation (PCS)-aneuploid phenotype. *Recql5* and *Blm* knockout led to very similar sister chromatid exchange (SCE) phenotypes and cancer predisposition phenotypes. However, *Recql5* suppresses tumorigenesis by minimizing the incident of gross chromosomal rearrangements (GCRs), whereas Blm reduces the tumor susceptibility in mice by suppressing the incidence of loss of heterozygosity (LOH). We found that Recql5 can suppress GCRs by preventing replication fork collapse in response to replication stress induced by the inactivation of topoisomerase I. Furthermore, in the vent that fork collapse has occurred, Recql5 can also function to inhibit the inappropriate initiation of HRR. Blm, on the other hand, suppressed LOH by promoting gene conversion over crossover once a homologous recombination repair (HRR) event has been initiated. Thus, Blm and Recql5 have non-redundant roles in regulating the process of HRR to maintain genome integrity. Importantly, both Blm and Recql5 are required to minimize the risks of oncogenic re-arrangements. In addition, our most recent data indicate that that Recql5/RECQL5 is specifically required for preventing the collapse of stalled DNA replication forks in response to treatments with topoisomerase I inhibitors. This new finding strongly suggest that Recql5/RECQL5 deficiency is not only a major causatic factor for tumorigenesis, but also an important determinant of sensitivity towards topoisomerase I inhibitors, an important class of anticancer agents. Therefore, human RECQL5 may represent an important biomarker for topoisomerase I inhibitors-based anticancer treatments, or perhaps even a potential target for developing new anticancer drugs.

Cooperative roles of vertebrate Fbh1 and Blm DNA helicases in avoidance of crossovers during recombination initiated by replication fork collapse; generation of a fish model of Bloom syndrome

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Fbh1 (F-box DNA helicase 1) orthologues are conserved from *Schizosaccharomyces pombe* to chicken and humans. Here we report the disruption of the *FBH1* gene in DT40 cells. Although the yeast *fbh1* mutant shows an increase in sensitivity to DNA damaging agents, *FBH1*$^{-/-}$ DT40 clones show no prominent sensitivity, suggesting that loss of *FBH1* might be substituted by other genes. However, *FBH1*$^{-/-}$ cells exhibit increase in both sister chromatid exchange and the formation of radial structures between homologous chromosomes without showing a defect in homologous recombination. This phenotype is reminiscent of *BLM*$^{-/-}$ cells, and suggests that Fbh1 may be involved in preventing extensive strand exchange during homologous recombination. In addition, disruption of *RAD54*, a major homologous recombination factor in *FBH1*$^{-/-}$ cells results in a marked increase in chromosome-type breaks (breaks on both sister chromatids in the same place) following replication fork arrest. Further, concurrent disruption of *FBH1* and *BLM* results in additional increases in both sister chromatid exchange and the formation of radial chromosomes. These data suggest that Fbh1 acts in parallel with the Bloom's syndrome helicase to control recombination mediated double strand break repair at replication blocks and reduce the risk of crossover.

The Medaka fish (*Oryzias latipes*) is an important model for developmental as well as comparative biology. Forward genetic approaches have already been shown to complement efforts in other model organisms such as the zebrafish, and the availability of the complete medaka genome sequence now prompts for tools to manipulate the encoded genes. We have generated target-selected medaka knockouts in the *Blm*, and *p53* genes, which were isolated from a cryopreserved library of 5,760 ENU-mutagenized fishes. Although virtually all *p53*$^{-/-}$ medaka fishes die due to tumorigenesis, *BLM*$^{-/-}$ medaka fishes can survive more than a year with no significant increase in the incidence of malignancy. Remarkably, spermatogenesis is impaired in *BLM*$^{-/-}$ fishes. *BLM*$^{-/-}$ medaka may be an excellent model to analyze the role of Blm in homologous recombination associated with crossover, since meiotic cell divisions can be followed with time in individual precursor cells in tissue culture.

Analysis of synthetic lethality phenotypes provides insights into functions of Drosophila BLM in maintaining genome stability

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Mammalian cells that lack the BLM helicase have high rates of sister chromatid exchange and elevated genome instability. We have been using *Drosophila melanogaster* as a model to study the cellular functions of BLM. Flies mutant for *mus309*, which encodes DmBLM, have defects in repair of DNA double-strand breaks and elevated mitotic crossing over, and mutant females are sterile due to an early embryonic requirement for maternal DmBLM1.

Analysis of double mutants has provided some insight into sources of genome instability. We recently examined synthetic lethality between *mus81*, which encodes a structure-specific DNA endonuclease, and *mus309*[2]. We found that synthetic lethality is associated with elevated P53-dependent apoptosis, and that removal of the Rad51 strand exchange protein partially suppresses both lethality and apoptosis. A *mus309* mutation that removes much of the N-terminus of DmBLM, but not the helicase domain, is viable with *mus81*, even though this mutation destroys the double-strand break repair function of DmBLM. We proposed a model in which: (1) some spontaneous DNA lesion, perhaps blocked replication forks, requires either MUS81 or DmBLM for repair, but repair does not involve RAD51; (2) in the absence of MUS81 and DmBLM, this lesion is converted into another lesion, perhaps a broken fork; (3) the second lesion requires RAD51 to convert it to an intermediate that is processed by DmBLM, but repair does not involve MUS81; (4) this intermediate is toxic and induces apoptosis if unprocessed.

We recently discovered synthetic lethality between *mus309* and *mus312*. *mus312* encodes a novel protein that is required to generate meiotic crossovers and is important in responding to DNA interstrand crosslinks[3]. The crossover function of MUS312 involves interaction with MEI 9, the *Drosophila* ortholog of the XPF endonuclease; however, *mei-9*; *mus309* mutants are viable, so the function of MUS312 responsible for synthetic lethality is independent of MUS312's function with MEI-9. The synthetic lethality of *mus309* and *mus312* is different than that described above because (1) *mus309 mus312* mutants have severe cell proliferation defects; (2) *mus309 mus312* mutants exhibit polyploidy and high rates of chromosome breakage; (3) ablating homologous recombination does not appear to rescue *mus309 mus312* lethality; and (4) the N-terminal deletion mutation if *mus309* is lethal with *mus312*, though it may ameliorate the phenotype somewhat. These results reveal a previously unknown function for DmBLM in maintaining genome stability. Ongoing characterization of this function and our studies of the vertebrate ortholog of MUS312 will be described.

1. McVey M, Andersen S, Broze Y, Sekelsky J. Multiple functions of DmBlm helicase in maintenance of genome stability. **Genetics** 2007; *176*:1979-1992

2. Trowbridge K, McKim KS, Brill S, Sekelsky J. Synthetic lethality in the absence of the Drosophila MUS81 endonuclease and the DmBlm helicase is associated with elevated apoptosis. **Genetics** 2007; *176*:1993-2001

3. Yıldız Ö, Majumder S, Kramer BC, Sekelsky J. Drosophila MUS312 interacts with the nucleotide excision repair endonuclease MEI-9 to generate meiotic crossovers. **Mol Cell** 2002; *10*:1503-1509

Role of SUMO in the absence of the yeast Sgs1 DNA helicase

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Yeast cells lacking the Sgs1-Top3-Rmi1 complex are viable, however their viability is dependent on several specific factors. We have explored the function of one of these factors, Slx5-Slx8, to gain insight into how a cell adapts to the loss of the BLM ortholog. Slx5-Slx8 is a heterodimeric Ub ligase that is required to suppress the accumulation of high molecular-weight SUMO conjugates. Interestingly, these conjugates appear to contain poly-SUMO chains. Structure-function analysis indicates that the Slx5-Slx8 Ub ligase contains multiple SUMO-binding domains consistent with the notion that it targets these highly SUMOylated proteins for destruction. To test this idea, we biochemically assayed Slx5-Slx8 Ub ligase activity using an auto-SUMOylated SUMO E3 ligase, Siz2, as a test substrate. In contrast to un-SUMOylated or multi-SUMOylated Siz2, substrates containing poly-SUMO conjugates were efficiently ubiquitinated by Slx5-Slx8. Surprisingly, the bulk of the Ub was directly conjugated to SUMO residues, not Siz2. The specific sites of ubiquitination by Slx5-Slx8 on SUMO are being determined and the consequences of eliminating these sites in cells lacking the Sgs1 DNA helicase will be presented. The results suggest that poly-SUMOylation plays a critical role in the absence of the BLM ortholog.

RecQ helicases, telomere recombination and maintenance

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Telomeres are a specialized structure at chromosome ends consisting of tandem repetitive DNA sequence [(TTAGGG)n in humans] and the associated proteins. Maintenance of the telomeric repeats, either by telomerase or by a recombination-mediated alternative lengthening of telomeres (ALT) mechanism, is essential for cellular immortalization. ALT is characterized by increased rates of recombination between telomeres of sister chromatids (T-SCE). In addition, there are similar frequencies of global homologous recombination in ALT and telomerase-positive cell lines. The molecular details of ALT pathway are largely unknown. Recently reports indicate that BLM and WRN are involved in telomere maintenance. BLM interacts with telomeric binding protein TRF1 and TRF2 to regulate telomeric DNA synthesis. WRN deficient cells display elevating T-SCE rates, which associated with greater immortalization for telomere maintenance via the ALT pathway. Moreover, functions of WRN on telomere maintenance are required for its helicase activity. Mismatch repair proteins mediate cellular proliferation in ALT cells and also interact and regulate BLM and WRN helicase activity. Telomeric single-stranded binding protein POT1 enhances BLM and WRN helicase activity *in vitro* and its isoforms are highly expressed in mismatch repair deficient cells, suggesting that POT1 may regulate BLM and WRN activity to maintain telomeric integrity associated with mismatch repair pathway *in vivo*. Taken together, RecQ helicases, telomere associated proteins and DNA repair factors may cooperate to the activation of the telomere maintenance mechanism in ALT cells through telomere recombination.

A multiprotein complex that maintains genome stability and is involved in Bloom syndrome and Fanconi Anemia

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Werner syndrome, Bloom syndrome (BS) and Rothmund-Thompson Syndrome are three human genetic diseases featuring genomic instability and increased risk for cancer. They are caused by mutations in three different helicases, WRN, BLM, and RecQ4, respectively, which belong to the same family of *E.Coli* RecQ helicases. To better understand how these RecQ helicases function *in vivo*, we used unbiased biochemical approaches to isolate complexes containing each of them and study their composition and activities. To date, we have isolated three distinct complexes that contain BLM, which were found to contain several published BLM-interacting proteins, including Topoisomerase IIIa, replication protein A, and MLH1. We also identified two proteins, named BLAP75 (RMI1) and RMI2, as novel components of BLM complexes essential for BLM to maintain genome stability (see the abstract by Guo et al.). Interestingly, one of the BLM complexes, termed BRAFT (for BLM, RPA, FA, and Topoisomerase IIIa), also contains eight Fanconi anemia (FA) complementation group proteins (FA). FA resembles BS in genomic instability and cancer predisposition. Fanconi anemia (FA) has recently become an attractive model to study breast cancer susceptibility (BRCA) genes, as three FA genes, *FANCD1, FANCN*, and *FANCJ*, are found to be identical to BRCA genes *BRCA2*, *PALB2*, and *BRIP1*. Our data suggest that BLM and FA proteins function in a common DNA damage response network that maintains genome integrity

SUMO modification of BLM and the regulation of anti-recombination

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The gene mutated in Bloom's syndrome (BS) BLM is a substrate for modification by small ubiquitin-like modifier (SUMO), and disruption of BLM SUMO modification induces the formation of γH2AX foci[1]. We hypothesized that SUMO modification might regulate BLM trafficking between its storage sites, the PML nuclear bodies (PML-NBs), and the putative sites for its action—damaged replication forks. To test this hypothesis, we stably expressed in BS cells a normal BLM (BLM cells) or a BLM double mutant (DM cells), with lysine to arginine substitutions at SUMO-acceptor sites 317 and 331, and treated the cells with hydroxyurea (HU), a reagent that stalls replication forks. Untreated BLM cells exhibited low levels of DNA repair foci (4.7 γH2AX foci/cell) whereas untreated DM cells exhibited four times the levels (20.5 γH2AX foci/cell). HU treatment stimulated γH2AX foci formation several fold in both BLM (31.1 γH2AX foci/cell) and DM (59.7 γH2AX foci/cell) cells, with an equivalent proportion of co-localized BLM-γH2AX foci in each. However, whereas BLM colocalization with γH2AX also decreased by half in BLM cells three hours after release from HU, BLM colocalization remained unchanged in DM cells, suggesting a kinetic defect in the trafficking of BLM DM back to the PML-NBs.

Consistent with the presence of excess γH2AX foci in HU-treated DM cells, the frequencies of HU-induced micronuclei formation and cell death were higher in DM compared with BLM cells. A synergistic killing effect by etoposide treatment was observed in HU-treated DM cells compared to HU-treated BLM cells, indicating the DM cells are hypersensitive to DNA damage encountered specifically during S phase. Unexpectedly, whereas BLM cells exhibited an approximately two-fold increase in HU-induced sister-chromatid exchanges (SCEs), there was no increase in HU-induced SCEs in DM cells. A failure to induce SCEs suggests a defect in homologous recombination repair that may be related in some way to the BLM DM trafficking defect in DM cells. Compared to BLM cells, DM cells are less able to engage homologous recombination to repair double strand breaks that arise at stalled replication forks, leading to increased micronuclei and cell death. We propose that SUMO modification of BLM promotes the repair of broken replication forks by relieving BLM's anti-recombination activity. Our model is that failure of SUMO modification of BLM leads to persistence of BLM in the repair foci and inhibition of RAD51-mediated replication fork restart.

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1. Eladad S, Ye TZ, Hu P, Leversha M, Beresten S, Matunis MJ, Ellis NA. Intra-nuclear trafficking of the BLM helicase to DNA damage-induced foci is regulated by SUMO modification. **Human Mol Genet** 2005; *14*:1351-1365

S. cerevisiae sgs1 mutants as a model system to develop strategies to suppress Bloom's syndrome (BS) phenotypes

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The BLM protein has been strongly implicated in homologous recombination repair (HRR). It is suspected that the abnormal processing of certain types of HRR intermediates might be responsible for many, if not all, BS phenotypes. Indeed, the elevated level of sister chromatid exchanges (SCEs), which is a diagnostic feature of BS, can be prevented by deletion of HRR genes in chicken DT40 *blm* cells. However, the molecular events that occur in BS cells that lead to elevated SCEs are not fully understood.

Since loss of key HRR proteins is likely to be lethal in human cells, we aim to develop strategies to alleviate the excessive SCEs in BS cells without causing cell death. To this end, we are utilizing the *S. cerevisiae* (budding yeast) *sgs1* mutant as a simple model system for BS. Sgs1 is the yeast ortholog of BLM and both of these proteins are closely associated with a type IA topoisomerase (Sgs1 with Top3 in yeast; BLM with hTOPOIIIα in humans), and an OB-fold nucleic acid binding protein (Rmi1 in yeast; BLAP75/RMI1 in humans), suggesting that the functions of these protein complexes are evolutionarily conserved. Furthermore, the *sgs1* mutant recapitulates many of the cellular defects observed in BS cells.

Consistent with the theory that BLM/Sgs1 are involved in some aspect of HRR, we are able to directly visualize unresolved HRR intermediates in *sgs1* mutants using 2-dimensional gel electrophoresis. Our ongoing aims are to develop strategies to genetically manipulate the accumulation and/or persistence of these abnormal structures in *sgs1* mutants. This involves two approaches: 1) mutation of genes that might genetically interact with SGS1, and 2) over-expression of proteins with defined DNA substrate specificities that might bypass the requirement for Sgs1. Due to the ease of genetic manipulation of yeast cells, we are able to rapidly screen through multiple targeted/rational approaches in this organism. In addition to facilitating characterization of the abnormal structures and how/why they arise in *sgs1* mutants, we aim to apply any successful strategies to BS cells. To date, we have identified at least two independent means to attenuate the abnormal structures detectable in *sgs1* mutants. Future studies should be aimed at investigating if these approaches similarly ameliorate BS cellular phenotypes.

Work in our laboratory is supported by the Bloom's Syndrome Foundation and Cancer Research UK.

Control of sister chromatid recombination in mammalian cells

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During the S and G2 phases of the cell cycle, DNA breaks are thought to be repaired preferentially by homologous recombination, using the neighboring undamaged sister chromatid as a template for repair ("sister chromatid recombination" – SCR). Circumstantial evidence suggests that several important cancer predisposition genes, including *BRCA1, BRCA2* and the Bloom's syndrome gene, *BLM*, collaborate to promote error-free SCR. To study mammalian SCR at a molecular level, we developed a recombination reporter for measuring SCR in response to a site-specific chromosomal double strand break. We have used it to identify genes that regulate SCR in mammalian cells and to characterize molecular mechanisms underlying individual SCR events. We are using this system to determine how *BLM* affects sister chromatid recombination, with the long-term goal of identifying new treatments that might suppress the genomic instability associated with loss of function of the Bloom's syndrome gene.

Poster No. 1

SSB: Organizational center and Achilles heel

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The C-terminus (Ct) of the single-strand DNA binding protein (SSB) from *Escherichia coli* forms a conserved binding site for numerous proteins involved in DNA replication, recombination, and repair[1]. SSB stimulates the enzymatic activities of several of its partner proteins, notably Exonuclease I[2] and RecQ helicase[3]. This stimulation is thought to be a result of the physical interaction between the proteins, which is mediated by the SSB-Ct. We have utilized NMR and X-ray crystallography to map the sites of interaction for the SSB-Ct in RecQ[3] and Exonuclease I[2], respectively. Mutation of SSB-Ct binding site residues in either protein results in loss of stimulation and/or inhibition by SSB. In addition to these biochemical studies, we have identified small molecule compounds that disrupt SSB interactions with Exonuclease I. With the essential nature of SSB protein interactions in bacteria, these studies could produce novel lead anti-bacterial compounds. We were able to identify several small molecules that disrupt this interaction. These compounds are potent anti-bacterial agents with little or no activity against eukaryotic cells.

1. Shereda RD, Lohman T, Cox, MM, Keck, JL. SSB as an organizer of genome maintenance. **Crit Rev Biochem Mol Biol** 2008; (in preparation)

2. Lu D, Keck JL. Structural basis of *E. coli* single-stranded DNA-binding protein stimulation of Exonuclease I. **Proc Natl Acad Sci** 2008; (in revision)

3. Shereda RD, Berstein DA, Keck JL. A central role for SSB in Escherichia coli RecQ DNA helicase function. **J Biol Chem** 2007; *282*:19247-19258

Poster No. 2

The death-by-recombination pathway promoted by E. coli RecQ

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The RecQ family of DNA helicases is highly conserved throughout evolution, and includes five human orthologues, three of which (BLM, WRN, and RECQ4) demonstrably suppress genomic instability and cancer. Our lab showed previously that RecQ promotes the net accumulation in cells of inter-molecular recombination intermediates (IRIs) that are toxic in the absence of RuvA or RuvB or RuvC and UvrD[1], proteins that resolve and prevent formation of IRIs, respectively. Excess IRI accumulation killed the cells by blocking chromosome segregation, and required RecA (RAD51 homologue), RecF (a RecA-loader), and RecJ (a 5' single-strand exonuclease proposed to interact with RecQ). This role of RecQ in promoting net IRI accumulation appears opposite to that of yeast Sgs1, Rqh1, human WRN, and probably BLM, but could apply to other human homologues as it appears to apply to a homologue in Arabidopsis[2]. RecQ might either promote formation of toxic IRIs, or alternatively, might block IRI resolution by a Ruv-independent pathway. A specific model in which RecQ-promoted IRI-initiation allows repair of replication-blocking lesions on the lagging strand was proposed[1]. We explored the effects of proposed RecQ-interacting proteins and report that promotion of the net accumulation of IRIs by RecQ is independent of either TopoIII or ExoI, both proposed to interact with RecQ. These results are compatible with an early role for RecQ in spontaneous IRI accumulation *in vivo*. Additionally, we find that Δ*rec*G Δ*uvr*D cells are inviable in a RecQ- and RecA-dependent manner (as Δ*ruv* Δ*uvr*D cells are), which is likely also to result from toxic IRI accumulation. As with Δ*ruv* Δ*uvr*D[1], Δ*rec*G Δ*uvr*D cells switched to a RecA$^+$ (toxic) state show chromosome-segregation defects that are dependent on RecQ, RecA, RecF, RecJ, and ExoI. These data could indicate either that the Ruv and proposed RecG IRI-resolution pathways have limited capacity, such that increasing the number of IRIs by UvrD-deficiency overwhelms either one when the other is absent, or alternatively, Ruv and RecG might recognize different types of RecQ-promoted IRIs, such that the absence of either pathway is toxic when both kinds of IRIs are in excess.

1. Magner DB, *et al*. RecQ promotes toxic recombination in cells lacking recombination intermediate-removal proteins. **Mol Cell** 2007; *26*:273-286

2. Hartung F, Suer S, Puchta H Two closely related RecQ helicases have antagonistic roles in homologous recombination and DNA repair in Arabidopsis thaliana. **Proc Natl Acad Sci USA** 2007; *104*:18836-18841

Functional role of BLAP75 in BLM/Topo IIIα-dependent Holliday junction processing

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The BLAP75 protein combines with the BLM helicase and Topo IIIα to form an evolutionarily conserved complex, termed the BTB complex, that functions to regulate homologous recombination (HR). BLAP75 binds DNA, associates with both BLM and Topo IIIα, and enhances the ability of the BLM-Topo IIIα pair to branch migrate the Holliday junction (HJ) or dissolve the double Holliday junction (dHJ) structure to yield non-crossover recombinants. Here we seek to understand the relevance of the biochemical attributes of BLAP75 in HJ processing. With the use of a series of BLAP75 protein fragments, we show that the evolutionarily conserved amino-terminal third of BLAP75 mediates complex formation with BLM and Topo IIIα and that the DNA binding activity resides in the carboxy-terminal third of this novel protein. Interestingly, the amino terminal third of BLAP75 is just as adept as the full-length protein in the promotion of dHJ dissolution and HJ unwinding by BLM-Topo IIIα. Thus, the BLAP75 DNA binding activity is dispensable for BTB complex's ability to process the HJ *in vitro*. Lastly, we show that a BLAP75 point mutant (K166A), defective in Topo IIIα interaction, is unable to promote dHJ dissolution and HJ unwinding by BLM-Topo IIIα. This result provides proof that the functional integrity of the BTB complex is contingent upon the interaction of BLAP75 with Topo IIIα.

Poster No. 4

BLAP15/RMI2 is a new component of the Bloom syndrome complex and essential for maintaining genome integrity

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Mutations in BLM cause Bloom syndrome (BS), which is a rare disorder characterized by growth retardation, genomic instability and cancer predisposition. BLM, BLAP75/RMI1 and Topo 3a form an evolutionarily conserved BLM complex and possess a double holiday junction (DHJ) dissolution activity[1,2]. The loss of DHJ dissolution activity is believed to be responsible for the genomic instability in BS cells. Here we report a new component of the BLM complex, named RMI2 (for RecQ-Mediated genome Instability 2). RMI2 stably associates with BLM, RMI1 and Topo 3a, and is required for the stability of the BLM complex. RMI2 also co-localizes with BLM and RMI1 in response to DNA damage, and is necessary for BLM and RMI1 to redistribute to nuclear foci in response to DNA damage. Depletion of RMI2 results in reduced phosphorylation level of BLM during mitosis. Importantly, chicken DT40 cells inactivated of RMI2 displays the hallmark feature of Bloom syndrome cells--an increased level of sister-chromatid exchange (SCE). Moreover, DT40 cells inactivated of both RMI2 and BLM exhibited an SCE level comparable to that of BLM-deficient cells. Our data demonstrate that RMI2 is a new essential component of the BLM complex, and participates in the same pathway as BLM in maintaining genome stability.

1. Wu L, Bachrati CZ, Ou J, Xu C, Yin J, Chang M, Wang W, Li L, Brown GW, Hickson ID. BLAP75/RMI1 promotes the BLM-dependent dissolution of homologous recombination intermediates. **Proc Natl Acad Sci USA**. 2006; *103*:4068-4073

2. Raynard S, Bussen W, Sung P. A double Holliday junction dissolvasome comprising BLM, topoisomerase IIIalpha, and BLAP75. **J Biol Chem** 2006; *281*:13861-13864

Poster No. 5

FANCJ helicase defective in Fanconia anemia and breast cancer unwinds G-quadruplex DNA to defend genomic stability

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FANCJ mutations are associated with breast cancer and genetically linked to the bone marrow disease Fanconi Anemia (FA). Genomic instability of FA-J mutant cells suggests FANCJ helicase functions in the replicational stress response. Putative helicases with sequence similarity to FANCJ in C. elegans (DOG-1) and mouse (RTEL) are required for polyG-tract maintenance, suggesting their involvement in resolution of alternate DNA structures that impede replication. Under physiological conditions, guanine-rich sequences spontaneously assemble into four-stranded structures (G4) that influence genomic stability. FANCJ unwound G4 DNA substrates in an ATPase-dependent manner. FANCJ G4 unwinding is specific since another Superfamily 2 helicase, RECQ1, failed to unwind all G4 substrates tested under conditions the helicase unwound duplex DNA. RPA stimulated FANCJ G4 unwinding, whereas the mismatch repair complex MSH2/6 inhibited this activity. FANCJ-depleted cells treated with the G4 interactive compound telomestatin displayed impaired proliferation, elevated apoptosis and DNA damage compared to siRNA control cells, suggesting that G4 DNA represents a physiological substrate of FANCJ. Although the FA pathway has been classically described in terms of interstrand cross-link (ICL) repair, the cellular defects associated with FANCJ mutation extend beyond the reduced ability to repair ICLs and involve other types of DNA structural roadblocks to replication.

Poster No. 6

Mechanism and substrate specificity of telomeric protein POT1 stimulation of the Werner syndrome helicase

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Loss of the RecQ helicase WRN protein causes the cancer-prone progeroid disorder Werner Syndrome (WS). WS cells exhibit defects in DNA replication, repair, and telomere preservation. The telomeric single stranded binding protein POT1 stimulates WRN helicase to unwind telomeric duplexes that are otherwise poorly unwound[1]. We reasoned that stimulation might occur by POT1 recruiting and retaining WRN on telomeric substrates during unwinding and/or by POT1 loading on partially unwound ssDNA strands to prevent re-annealing. We tested these possibilities by using substrates with POT1 binding sequences in the single stranded tail, duplex region or both regions. POT1 binding to ssDNA tails did not alter WRN unwinding of non-telomeric duplexes, or WRN amounts bound to the telomeric ssDNA. However, POT1 pre-loading inhibited WRN activity on telomeric duplexes with a protruding 3' ssDNA tail, but stimulated WRN unwinding of forked telomeric duplexes with two tails. This indicates that POT1 interaction with the ssDNA/dsDNA junction regulates WRN helicase. Furthermore, POT1 did not alter the ratio of telomeric versus non-telomeric forks unwound by WRN, indicating POT1 does not enhance WRN retention on telomeric forks during unwinding. Collectively, these data suggest POT1 promotes the apparent processivity of WRN helicase by maintaining partially unwound strands in a melted state, rather than serving as a WRN clamp.

1. Opresko PL, Mason PA, Podell ER, Lei M, Hickson ID, Cech TR, Bohr VA. POT1 stimulates RecQ helicases WRN and BLM to unwind telomeric DNA substrates. **J Biol Chem** 2005; *280*: 32069-32080

Poster No. 7

Functional characterisation of the human RECQL5 helicase

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Damage of DNA is a frequent event and major challenge to all cells. Accumulation of DNA damage leads to genomic instability, which is a general feature of cancer cells.

The RecQ family of DNA helicases is highly conserved and comprises five members in humans namely RECQL, BLM, WRN, RECLQ4 and RECQL5. Mutations in *BLM, WRN* and *RECQ4* cause Bloom's, Werner's and Rothmund-Thomson syndrome, respectively. These autosomal recessive disorders are characterised by genomic instability, cancer predisposition and premature aging. Although no genetic disorder for *RECQ5* deficiency has yet been described, mouse Recql5-deficient cells display chromosomal abnormalities and elevated levels of sister chromatid exchanges.

Since RecQ helicases have been implicated in the resolution of potentially deleterious DNA structures that can arise during DNA replication, we are investigating the contribution of RECQL5 to replication fork integrity following DNA damage.

Poster No. 8

Response of Rothmund-Thomson syndrome fibroblasts to genotoxic agents

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Background: Rothmund-Thomson syndrome (RTS) is a member of the RECQ family of disorders that includes Bloom (BLM) and Werner (WRN) syndromes, all of which are all characterized by increased cancer predisposition. Two-thirds of patients with RTS have deleterious mutations in the *RECQL4* gene, and genotype-phenotype analysis has shown that presence of mutations correlates with risk of osteosarcoma, a primary malignant bone cancer. The exact function of RECQL4 has not been clearly delineated, but it has been shown in a Xenopus model to play a role in initiation of replication. Previous studies have shown that BLM and WRN-deficient cells demonstrate increased sensitivity to hydroxyurea (HU), camptothecin (CPT), and 4-nitroquinoline 1-oxide (4NQO). Less is known about the sensitivity of RECQL4-deficient cells to these and other genotoxic agents. Cells from RTS patients have in a few cases been reported to have increased sensitivity to DNA damaging agents such as ultraviolet (UV) radiation, but results have been somewhat conflicting. We undertook this study to examine the response of RECQL4-deficient primary cells from RTS patients to a broad panel of genotoxic agents.

Methods: Primary untransformed fibroblasts from ten RTS subjects were used for this study along with wild type (WT) and appropriate positive controls. All subjects were enrolled in an IRB-approved study at Baylor College of Medicine and provided informed consent for participation. Clonogenic survival assays were performed testing response of RTS and control cells to the following agents: HU, CPT, 4NQO, cisplatin (CDDP), doxorubicin (DOX), UV and ionizing radiation (IR). Dose-response curves for studying the sensitivity to agents were fitted by the generalized linear regression model, and statistical analyses were performed using the statistical packages R and STATA.

Results: Our results showed that primary fibroblasts from RTS patients carrying two deleterious *RECQL4* mutations, compared to WT fibroblasts, demonstrated increased sensitivity to HU, CPT, and DOX, modest sensitivity to UV, IR, and CDDP, and resistance to 4NQO. In the case of HU and CPT, these responses were intermediate between WT (negative) and BLM or WRN (positive) controls. However, the response to 4NQO was distinctly different from BLM and WRN.

Conclusions: Because HU, CPT and DOX exert their effects primarily during S phase, these results support a greater role for the RECQL4 protein in DNA replication as opposed to repair of exogenous damage cause by other agents such as UV and IR. These findings may also have implications for the clinical management of patients with RTS.

Poster No. 9

Interaction of the BLM helicase and topoisomerase IIα modulates helicase activity and protects against chromosome breaks

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Bloom's syndrome (BS) is an autosomal recessive disorder in which affected individuals show pre- and post-natal growth retardation, sun-sensitivity, cancer susceptibility, immunodeficiency, and male infertility. BS cells are characterized by a high rate of spontaneous sister chromatid exchanges (SCE) and chromosome breakage. Known interactions between recQ-like helicases and topoisomerases, suggest their cooperation in DNA transactions such as fork progression and recombinational repair, as in *Saccharomyces cerevisiae*, the interaction between the recQ-like helicase Sgs1 and topoisomerase II is required for proper chromosome segregation. Topoisomerase IIα removes catenation or tangles following DNA replication, assuring proper chromosome segregation in mitosis. Our current work demonstrates that BLM and topoisomerase IIα co-immunoprecipitate from asynchronous human and mouse cells, and co-localize in many small punctate foci. These proteins also co-immunoprecipitate in a telomere-specific complex in immortalized cells using the alternative lengthening of telomere (ALT) mechanism to elongate telomere ends. When cells are treated with the topoisomerase IIα catalytic inhibitor, ICRF-193 that prevents ATP- and DNA- binding of topoisomerase IIα, both proteins co-immunoprecipitate, but co-localize in a greatly reduced number of large foci. Cell cycle arrest experiments using hydroxyurea (S-phase), ICRF-193 (late S-phase-G2-M), olomucine (G2-M) and colcemid (M-phase) show that the proteins co-immunoprecipitate in late S, G2 and M-phases. *In vitro*, expression analyses demonstrate that the N-terminus of BLM is required for co-immunoprecipitation with topoisomerase IIα. *In vitro* DNA unwinding activity of BLM on a 3' overhang substrate is enhanced by topoisomerase IIα at low concentrations of BLM (5 nM) but not at high concentrations (30 nM); using a telomere-like substrate, helicase activity is enhanced by topoisomerase IIα at high concentrations of BLM (30 nM). Conversely, BLM does not affect *in vitro* decatenation of DNA by topoisomerase IIα. SiRNAs specific for BLM and topoisomerase IIα, and comet assays were used to determine how the loss of one or both proteins affect chromosome breakage. Cells deficient in BLM, topoisomerase IIα, or both BLM and topoisomerase IIα are characterized by increased chromosome breakage, suggesting that BLM and topoisomerase IIα function in the same pathway in the cell to protect against chromosome breaks.

Poster No. 10

Novel phosphorylation sites in BLM regulate localization to stalled replication forks and helicase activity

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BLM is a RecQ-like helicase with 3'-5' ATP-dependent helicase activity required for chromosome stability. Published work demonstrates that post-translational modification of BLM is cell cycle-specific, and that phosphorylation plays an important role in regulating its function and localization. Twenty two putative phosphorylation sites within BLM were identified using germline mutation analysis of *BLM*, bioinformatic analysis; mass spectrometry and literature review, and were mutated using site-directed mutagenesis to phosphomimetic and non-phosphorylated forms. To date, replication fork regression assays have identified seven sites, S186, T547, T766, S801, S1290, S1296, and S1386, that may regulate BLM activity at stalled replication forks. Aspartic acid modifications that mimicked phosphorylation at S186, T547, S1290, S1296, and S1386 showed greater fork regression activity than wild-type BLM, while aspartic acid modification at T766 or S801 resulted in loss of activity. Unwinding assays using a DNA duplex with a 3' overhang suggest that phosphorylation at S186, T547 or S1386 activates BLM unwinding, and that de-phosphorylation diminishes unwinding. Additionally, modification at T766 or S801 abolishes the unwinding activity of BLM. Assessing the ability of phosphorylation mutants in GFP-fusion vectors to correct the increased micronuclei formation exhibited by BS cells shows that T547A cannot correct, whileT547D mutation can recover micronuclei numbers similarly to wild-type BLM. These results suggest that phosphorylation of S186, T547, T766, S801 and S1386 may be important for regulating the overall helicase function of BLM, but that phosphorylation of S1290 and S1296 may control a replication fork-specific function of BLM. It is known that ATR is required for BLM localization to stalled replication forks and that it phosphorylates BLM at T99 and T122, although this is not required for BLM localization to stalled forks. These data suggest there are other putative ATR phosphorylation sites within BLM or a BLM protein partner localizes BLM in response to ATR activation. We tested this by observing the ability of mutants S1290A, S1290D, S1296A, and S1296D to localize to stalled replication forks after hydroxyurea (HU) treatment. These data show that S1290A and S1296A exhibit altered distribution after HU treatment compared to wild-type BLM and suggest that these sites may be critical for controlling localization of BLM after HU-induced DNA damage.

Poster No. ii

Characterizing the genetic requirements of hyper-recombination in Drosophila BLM mutants

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Meiotic crossovers are programmed and important for normal chromosome segregation. In contrast, mitotic crossovers are potentially detrimental; inappropriate crossing over in proliferating cells is associated with gross genome instability, including chromosome rearrangements and chromosome loss. Thus, mitotically dividing cells have well-conserved mechanisms to prevent crossovers. Formation of mitotic crossovers is blocked, at least in part, by the RecQ-family helicase BLM; cells lacking BLM have increased genomic instability, including increased crossing over between sister chromatids and homologous chromosomes.

The gene *mus309* encodes the *Drosophila* ortholog of BLM. *mus309* mutants have elevated mitotic crossovers in germline and somatic cells[1]. The crossovers can be increased further by DNA-damaging treatments, including ionizing and UV radiation[1]. Using candidate genes with known roles in meiotic recombination and/or DNA repair, we are determining the genetic requirements for mitotic crossovers in *Drosophila mus309* mutants. This will further our understanding of how BLM functions to maintain genome stability.

Not unexpectedly, we've found that proteins which function early in recombination are important for the production of mitotic crossovers in *mus309* mutants. Crossovers in *mus309* mutants are severely reduced by mutation of genes that encode the strand-invasion proteins DmRAD54 and DmRAD51.

We've also tested exonucleases as candidates for cutting recombination intermediates to produce crossovers, including MEI-9 and MUS81. The ortholog of XPF/Rad1, MEI-9 has roles in meiotic crossovers, interstrand crosslink repair, and nucleotide excision repair (NER) in *Drosophila*[2].MUS81 is required for a subset of meiotic crossovers in some organisms, including *S. cerevisiae*, and has a role in repairing blocked/damaged replication forks[3]. *mei-9* and *mus81* single mutants have wild-type crossover levels. *mei-9 mus309* and *mus81 mus309* double mutants have highly increased crossover levels compared to *mus309* single mutants, as does the mei9 *mus81 mus309* triple mutant. Thus, the nucleases MEI-9 and MUS81 are not individually required for producing crossovers in the *mus309* mutant background, nor are they acting redundantly. Further, the increased crossing over in *mei-9 mus309* mutants is ablated when a separation-of-function allele of *mei-9* defective in meiotic crossovers but proficient in NER is used. This suggests that unrepaired damage causes increased crossovers in the *mei-9 mus309* double mutants. By analogy, replication-associated damage that is unrepaired in the absence of MUS81 may be responsible for the crossover increase in *mus81 mus309* double mutants.

Additional candidate genes are currently being investigated.

1. McVey M, Andersen SL, Broze III GJ, Sekelsky JJ. Multiple functions of Drosophila Blm helicase in maintenance of genome stability. **Genetics** 2007; *176*:1979-1992

2. Boyd JB, Golino MD, Setlow RB. The mei-9 alpha mutant of Drosophila melanogaster increases mutagen sensitivity and decreases excision repair. **Genetics** 1976; *84*:527-544

3. Haber JE, Heyer WD. The fuss about Mus81. **Cell** 2001; *107*: 551-554

Poster No. 12

Genetic analysis of homologous recombination repair pathways in mouse embryonic stem cells

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Genomic instability can be found in cells isolated from Bloom's syndrome (BS) individuals. BS is an autosomal recessive disorder associated with cancer predisposition. Evidence suggests that BLM plays roles in homologous recombination (HR), a process that is important for the repair of DNA double strand breaks (DSBs) and damaged replication forks. We are studying the interactions between BLM and Rad54, one of the central players in HR. Rad54 is involved in the stabilization of Rad51 nucleoprotein filaments during DNA repair. The *blm* and *rad54* genes have been disrupted individually and together in mouse embryonic stem cells. By treating the single and double mutant cells with various DNA damaging agents, we have shown that the double mutant shows hypersensitivity to Cisplatin and Camptothecin. However, disruption of *blm* can rescue the MMC sensitivity of a *rad54* mutant, as well as lower the level of DSBs in the double mutant treated with MMC. In addition, *rad54* mutation partially suppresses the elevated level of SCEs in *blm* cells, indicating that the hyper-SCE phenotype of *blm* mutant cells is at least partially due to excess HR.

Poster No. 13

Mechanisms of gene conversion in wild-type and Msh2 mutant mammalian cells

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Accurate and efficient repair of damaged DNA is necessary to maintain genomic integrity. Repair of damaged DNA is dependent on many factors, some of which include proteins found in a multi-subunit complex called BRCA1-associated genome surveillance complex (BASC). These include components of the mismatch repair (MMR) machinery, as well as ATM, BLM and MRE11[1]. Loss of these factors results in a variety of human diseases that are often linked to chromosomal instability.

Particularly toxic lesions are double-strand breaks (DSBs), which can arise from environmental factors as well as during normal cellular processes. DSBs can be repaired by two strategies: conservative homologous recombination (HR) or error-prone nonhomologous end joining. Repair by HR may occur by a pathway in which crossovers and noncrossover gene conversions are alternative outcomes or through synthesis-dependent strand annealing (SDSA) in which noncrossover gene conversions predominate. Here we have analyzed gene conversion events in MMR-defective *Msh2*$^{-/-}$murine ES cell lines to extend studies previously reported in this lab and others[2]. A novel repair substrate with 1.5% sequence divergence was developed to finely map gene conversion tracts after induction of a single DSB. We found that MSH2 substantially suppresses HR using a donor with 1.5% of sequence divergence. Additionally, analyzing repair events in the MMR defective cells allowed us uncovered heteroduplex DNA (hDNA) that would have otherwise undergone mismatch correction. Using this approach, we found that a significant portion of gene conversion tracts in *Msh2*$^{-/-}$cells are uncorrected, although conversion adjacent to the DSB is often complete. We also found that the donor sequence remains unaltered in 100% of all gene conversion events analyzed from both wild type and *Msh2*$^{-/-}$cells, suggesting that SDSA contributes to most, if not all gene conversion events in mammalian systems. Moreover, there was no indication of mutagenesis during HR of the repaired gene. Lastly, nearly all gene conversion events in both wild type and *Msh2*$^{-/-}$mutants were associated with conversion on only one side of the break, suggestive of one-end invasion resulting in gene conversion.

This work provides more evidence that MMR machinery suppresses illegitimate recombination and demonstrates novel information regarding the mechanism of gene conversion. Because of the association with the loss of BASC complex proteins and genomic instability, it is of interest to determine the roles of other components of the BASC complex in HR, in particular BLM.

1. Fukimata M, Liu J. Chromosomal breakage syndromes and the BRCA1 genome surveillance complex. **Trends Mol Med** 2001; *7*:560-565

2. Elliott B, Jasin M. Repair of double-strand breaks by homologous recombination in mismatch repair-defective mammalian cells. **Mol Cell Biol** 2001; *21*:2671-2682

Poster No. 14

Distinct roles of chromatin-associated factors MDC1 and 53BP1 in mammalian double strand break repair

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Phosphorylated histone H2AX ("g-H2AX") recruits MDC1, 53BP1 and BRCA1 to chromatin near a double strand break (DSB) and facilitates efficient repair of the break. It is unclear to what extent g-H2AX associated proteins act in concert and to what extent their functions within g-H2AX chromatin are distinct. We addressed this question by comparing the mechanisms of action of MDC1 and 53BP1 in DSB repair (DSBR)[1]. We find that MDC1 functions primarily in homologous recombination/sister chromatid recombination, in a manner strictly dependent upon its ability to interact with g-H2AX but, unexpectedly, not requiring recruitment of 53BP1 or BRCA1 to g-H2AX chromatin. In contrast, 53BP1 functions in XRCC4-dependent non-homologous end-joining, likely mediated by its interaction with dimethylated lysine 20 of histone H4 but, surprisingly, independent of *H2AX*. These results suggest a specialized adaptation of the "histone code", in which distinct histone tail-protein interactions promote engagement of distinct DSBR pathways. I am currently testing this hypothesis by analyzing DSBR functions in cells lacking the two major histone H4 lysine 20 methyltransferases.

1. Xie A, Hartlerode A, Stucki M, Odate S, Puget N, Kwok A. Nagaraju G, Yan C, Alt FW, Chen J, Jackson SP, Scully R. Distinct roles of chromatin-associated proteins MDC1 and 53BP1 in mammalian double-strand break repair. **Mol Cell** 2007; *28*:1045-1057

Poster No. 15

RecQ helicases and their role in oxidative DNA damage repair at the telomere

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The RecQ helicases WRN and BLM play important roles in mediating DNA damage repair and there is evidence that this occurs within telomeric regions through interaction with telomeric proteins. To determine the functional relationships between the various telomere interacting proteins and oxidative DNA damage repair mechanisms, as well as determine the role RecQ helicases have in these processes, we have performed a variety of *in vitro* activity assays with substrates designed to better mimic telomeric D-loop structures. Our initial results are in concurrence with previously published data showing that POT1 and RPA modulate WRN helicase and exonuclease activities on undamaged telomeric substrate *in vitro*. Here we show that telomeric D-loops with 8-oxoguanine lesions are unwound by BLM and WRN, but that WRN exonuclease activity is completely abrogated. Most interestingly, our results indicate that both WRN and BLM helicases unwind Dloop with 8-oxoguanine more efficiently than D-loop without these lesions. Furthermore, we show that POT1 binds over 8-oxoguanine lesions on single stranded telomeric DNA and inhibits WRN exonuclease activity, as well as OGG1 incision activity on this substrate. Together, this data provides further support for WRN, BLM and possibly other RecQ helicases in mediating oxidative DNA damage repair at telomeric DNA.

Poster No. 16

A collaborative role of Werner syndrome protein and ATR in replication stress induced double strand break (DSB) repair

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Double strand breaks (DSBs) are highly cytotoxic lesions, which arise during exposure to ionizing radiation, replication stress and meiotic recombination. Defects in repairing these lesions are associated with human chromosome fragility syndromes such as Werner syndrome (WS), Nijmegen breakage syndrome (NBS), ataxia telangiectasia (AT), and ataxia telangiectasia-like disorder (ATLD). WRN (Werner syndrome protein), a RecQ DNA helicase, and ataxia telangiectasia and Rad3-related protein (ATR) are implicated in the resolution of stalled replication forks (Holliday junctions) and homologous recombination repair (HR). Werner syndrome protein participates in a complex with ATR, RAD51, RAD54 and RAD54B in response to replication arrest[1]. Beside WRN and ATR, in a common pathway, play a crucial role in preventing chromosome breakage at fragile sites during replication[2]. However, the precise roles of WRN and ATR in the repair of replication stress induced DSB are not understood and the molecular mechanisms of DSB repair at the initial processing stage has not yet been established. We investigated the involvement of a collaborative role of WRN and ATR in the signaling response to replication stress induced DSB. Cells without WRN showed increased accumulation of camptothecin induced DSB. Despite having more DSB WRN depleted cells have significantly less ATR mediated phosphorylation of H2AX, RPA32 and Chk1. Moreover, we have demonstrated that Chk1 inhibition mediated ATR activation is abrogated in WRN depleted cells. This study suggests that a defective optimal ATR activation might, at least in part, explains sensitivity of the WRN-deficient cells towards replication-mediated double-strand breaks.

1. Otterlei M, Bruheim P, Ahn B, Bussen W, Karmakar P, Baynton K, Bohr VA. Werner syndrome protein participates in a complex with RAD51, RAD54, RAD54B and ATR in response to ICL-induced replication arrest. **J Cell Sci** 2006; *119*:5137-5146

2. Pirzio LM, Pichierri P, Bignami M, Franchitto A. Werner syndrome helicase activity is essential in maintaining fragile site stability. **J Cell Biol** 2008; *180*:305–314

Poster No. 17

Multiplex analysis of TRF2 modulation of WRN helicase/ exonuclease dissociation of HJ DNA

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Telomeres are protein-DNA complexes that prevent chromosome ends from being recognized as double strand breaks. The telomere-binding protein TRF2 protects telomeres against unwanted processing by homologous recombination *in vivo*. TRF2 specifically binds telomeric duplex DNA, and has a basic N-terminal domain that binds four-way junctions regardless of sequence[1]. TRF2 also interacts with Werner Syndrome protein (WRN) and stimulates its helicase activity[2]. TRF2 thus has dual functions in that it modulates WRN helicase/exonuclease activity but also protects HJ DNA against unwanted dissociation. We hypothesize that TRF2 may provide a type of molecular "switch", providing both protection to recombination-like structures at telomeres and also modulating WRN-driven processing of such structures in a concentration-dependent manner.

We are using multiplex labeling of HJ DNA arms with fluorophores to simultaneously track the fates of all four strands in HJ DNA displacement by WRN protein. We are able to differentiate between the components of three-way, duplex and exonuclease species. We have found that both WRN helicase and exonuclease simultaneously produce a mixture of well-defined populations of duplex and single-stranded species, and that HJ DNA dissociation is much less efficient with helicase alone than in conjunction with exonuclease. We are currently studying the effect of TRF2 in modulating WRN helicase/exonuclease on mixed-sequence HJ DNA.

1. Fouche N, Cesare AJ, Willcox S, Ozgur S, Compton SA, Griffith JD. The basic domain of TRF2 directs binding to DNA junctions irrespective of the presence of TTAGGG repeats. **J Biol Chem** 2006; *281*:37486-37495

2. Opresko PL, von Kobbe C, Laine JP, Harrigan J, Hickson ID, Bohr VA. Telomere-binding protein TRF2 binds to and stimulates the Werner and Bloom syndrome helicases. **J Biol Chem** 2002; *277*:41110-41119

Poster No. 18

Functional analysis of the BLM helicase in RNA polymerase-I transcription and characterization of its helicase activity on RNA:DNA substrates

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In the autosomal recessive disorder Bloom's Syndrome (BS), the *BLM* locus is mutated leading to loss of activity of the BLM helicase. Somatic cells from BS patients exhibit excessive sister chromatid exchange (SCE) and are hypermutable. BLM localizes to nucleoli, and such localization is crucial for maintenance of genomic stability[1]. Since nucleoli contain ribosomal DNA (rDNA) repeats, and BLM specifically localizes to nucleoli during S-phase and G2-phase of the cell cycle[2], the precise time during which rDNA transcription occurs[3], we investigated a putative function for BLM in RNA polymerase-I transcription. We report that nucleolar accumulation of BLM requires ongoing RNA polymerase-I transcription, as well as integrity of single-stranded RNA. Additionally, the rate of ribosomal RNA synthesis is decreased in BS lymphoblastoid cell lines, which suggests a possible role for BLM in accelerating RNA polymerase-I transcription. *In vitro*, BLM binds strongly to single-stranded RNA and unwinds RNA:DNA hybrid duplexes, but not RNA:RNA duplexes. We propose that nucleolar BLM functions to maintain genomic stability of the GC-rich rDNA by destabilizing aberrant RNA:DNA structures that may form during RNA polymerase-I transcription.

1. Yankiwski V, Noonan JP, Neff NF. The C-terminal domain of the Bloom syndrome DNA helicase is essential for genomic stability. BMC **Cell Biology** 2001; *2*:11

2. Yankiwski V, Marciniak RA, Guarente L, Neff NF. Nuclear structure in normal and Bloom syndrome cells. **Proc Natl Acad Sci USA** 2000; *97*:5214-5219

3. Ayrault O, Andrique L, Fauvin D, Eymin B, Gazzeri S, Seite P. Human tumor suppressor p14ARF negatively regulates rRNA transcription and inhibits UBF1 transcription factor phosphorylation. **Oncogene** 2006; *25*:7577-7586

Bloom's syndrome: from genes to molecules to cells to people.

(Cover illustrations –clockwise from the top left)

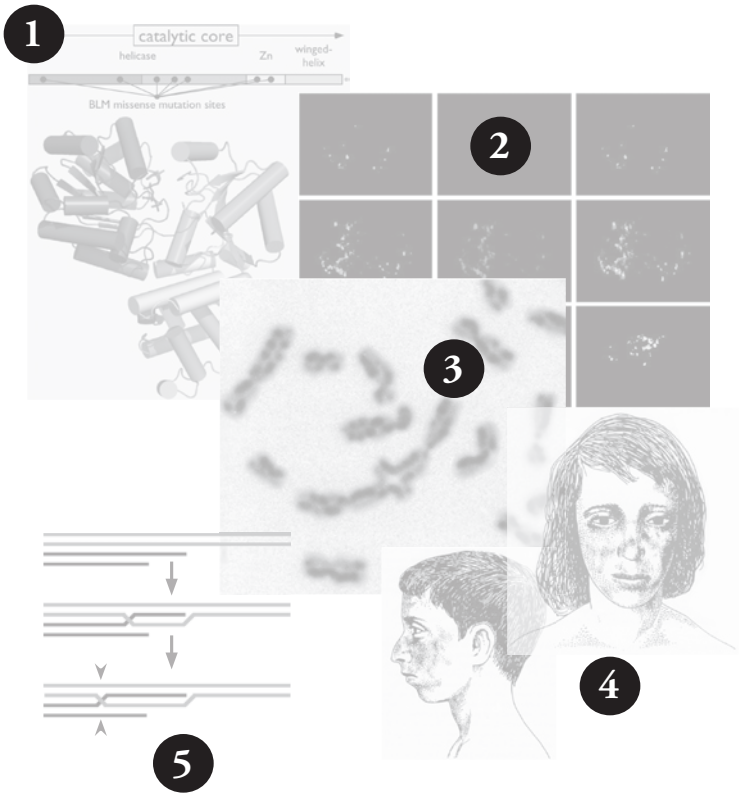
1. A diagrammatic representation of *E. coli* RecQ helicase and the locations at which known missense mutations identified in persons with Bloom's syndrome are mapped *(Courtesy of James L. Keck, University of Wisconsin).*

2. Indirect immunofluorescence images showing the co-localization of BLM protein (green) with phosphorylated H2AX (red), which marks DNA double-strand breaks, in normal human cells treated with bleomycin (middle) or hydroxurea (bottom) *(Figure 6 from Davalos and Campisi, Journal of Cell Biology 162:1197-209, 2003).*

3. Metaphase chromosomes from a Bloom's syndrome lymphocyte showing the abnormally elevated numbers of sister-chromatid exchanges and a homologous chromatid interchange configuration, also known as a quadriradial or Qr *(cover from Cell, November 17, 1995).*

4. Composite drawings from two photographs of children with Bloom's syndrome, to demonstrate the characteristic head configuration and facies *(drawing by Bunji Tagawa; reproduced from Hospital Practice 8:93-104, 1973).*

5. Line drawing depicting steps to restart a broken replication fork and a proposed mechanism for the generation of a sister-chromatid exchange.

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