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Meeting report

Bloom's syndrome workshop focuses on the functional specificities of RecQ helicases

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ABSTRACT

Human cells express five DNA helicases that are paralogs of *Escherichia coli* RecQ and which constitute the family of human RecQ helicases. Disease-causing mutations in three of these five human DNA helicases, *BLM*, *WRN*, and *RECQL4*, cause rare severe human genetic diseases with distinct clinical phenotypes characterized by developmental defects, skin abnormalities, genomic instability, and cancer susceptibility. Although biochemical and genetic evidence support roles for all five human RecQ helicases in DNA replication, DNA recombination, and the biological responses to DNA damage, many questions concerning the various functions of the human RecQ helicases remain unanswered. Researchers investigating human and non-human RecQ helicases held a workshop on May 27–28, 2008, at the University of Chicago Gleacher Center, during which they shared insights, discussed recent progress in understanding the biochemistry, biology, and genetics of the RecQ helicases, and developed research strategies that might lead to therapeutic approaches to the human diseases that result from mutations in RecQ helicase genes. Some workshop sessions were held jointly with members of a recently formed advocacy and support group for persons with Bloom's syndrome and their families. This report describes the outcomes and main discussion points of the workshop.

1. Introduction

Bloom's syndrome (BS) is a rare autosomal recessive disorder characterized by developmental defects, sun sensitivity, and cancer susceptibility. BS is caused by mutations in *BLM*, which encodes one of the five human RecQ family 3' to 5'-DNA helicases. The other human RecQ helicases are *WRN*, *RECQL*, *RECQL4*, and *RECQL5*. Defects in *WRN* cause Werner syndrome (WS), a disease characterized by symptoms resembling premature aging, while defects in *RECQL4* can cause three human diseases: Rothmund-Thomson, RAPADILINO, and Baller-Gerold syndromes. RecQ helicases are implicated in the interplay between replication stress and repair of DNA damage by homologous recombination (HR). These rare human genetic diseases provide an opportunity to gain insight into the mechanisms by which defects in DNA replication, HR, and DNA repair contribute to aging and cancer susceptibility.

Abbreviations: ALT, alternative lengthening of telomeres; BS, Bloom's syndrome; CPT, camptothecin; dsDNA, double-stranded DNA; DSB, double-strand break; ES cells, embryonic stem cells; G4, G quartet; G4P, G4 DNA-forming potential; HR, homologous recombination; HU, hydroxyurea; ICL, interstrand cross-link; MMS, methylmethane sulfonate; ssDNA, single-stranded DNA; SCE, sister-chromatid exchange; SCR, sister-chromatid recombination; WS, Werner syndrome.

2. Workshop overview

Researchers investigating human and non-human RecQ helicases held a workshop¹ May 27–28, 2008, at the University of Chicago Gleacher Center, during which they shared insights and discussed recent progress in understanding the biochemistry, biology, and genetics of the RecQ helicases. Workshop participants also discussed research strategies that might lead to therapeutic approaches to the human diseases resulting from defects in these enzymes. The workshop included eight scientific sessions, two poster sessions, and an evening reception held jointly with Bloom's Connect (<http://www.bloomsconnect.org>), a recently formed advocacy and support group for persons with BS and their families. This report describes the outcomes and main discussion points of the workshop (Box 1).

¹ The workshop "Molecular and Clinical Mechanisms in Bloom's Syndrome and Related Disorders" held May 27–28, 2008, at the University of Chicago Gleacher Center was co-organized by Nathan Ellis (University of Chicago), Vilhelm A. Bohr (National Institute of Aging, NIH), and Curtis C. Harris (National Cancer Institute). The workshop was sponsored by the National Institutes of Health Office of Rare Diseases, National Cancer Institute, National Institute of Aging, University of Chicago Cancer Research Center, The Bloom's Syndrome Foundation, and Bio-Rad Laboratories.

Box 1. Key Workshop Outcomes

Replication/recombination and the response to DNA damage

BLM-defective cells demonstrate slow and asymmetrical replication fork movement and spontaneously activate DNA damage checkpoints in the absence of exogenous DNA damage. Thus, BS clinical symptoms may reflect profound defects in DNA replication.

WRN-deficiency causes a 25-fold decrease in the number of viable daughter cells that undergo mitotic gene conversion. Although WRN-deficient cells initiate mitotic recombination, they fail to resolve recombination intermediates and generate viable recombinant daughter cells.

The rate of CPT-induced chromosomal aberrations is higher in mouse *Recq15*^{-/-} but not in mouse *Blm*^{-/-} cells. *Recq15*^{-/-} cells are also sensitive to CPT-induced loss of survival, whereas *BLM*^{-/-} cells are not. Thus, *Recq15* and *Blm* have non-redundant roles in the response to CPT-induced DNA damage.

Cancer susceptibility

Haploinsufficiency for *Blm* predisposes to MLV-induced lymphoma and to colorectal cancer in *APC*^{min} doubly heterozygous mice. The cancer susceptibility of human carriers of defective alleles of *BLM* requires further study.

Recq15^{-/-} mouse ES cells have a higher frequency of sister-chromatid exchange than wild type cells and *Recq15*^{-/-} mice die at a younger age than wild type mice, due to increased susceptibility to lethal sporadic cancer. Because *Recq15*^{-/-} cells are uniquely hypersensitive to CPT-induced damage, expression and function of *Recq15* may be an important predictor of the outcome of CPT-treatment for cancer patients.

Post-translational modification of RecQ helicases and other target proteins

BLM includes a SUMO interaction motif and four preferred sumoylation sites. Sumoylation of BLM may play key role in regulating trafficking of BLM to and from DNA damage-induced foci.

The subcellular localization of RECQL4 is regulated by p300-mediated acetylation of a lysine-rich motif. Acetylated RECQL4 is refractory to nuclear import and is sequestered in the cytoplasm, where it plays an as yet undetermined biological function.

Yeast mutants lacking Sgs1 require Sxl5 and Sxl8 for viability. Sxl5 and Sxl8 are subunits of an unusual heterodimeric E2-ubiquitin ligase that preferentially ubiquitinates the terminal SUMO moiety in polysumoylated target proteins *in vitro*.

Protein–protein interactions

BLM forms a functional core complex that includes RMI1 (BLAP75), RMI2 (BLAP18), and TopoIII α . The BLM complex interacts with the Fanconi Anemia core complex to form a multiprotein supercomplex including as many as 20 polypeptides.

E. coli RecQ binds to and is stimulated by *E. coli* SSB via its winged helix subdomain. This functional role of the winged helix subdomain is conserved in mammalian BLMs.

BLM disrupts the RAD51-ssDNA filament *in vitro*, suggesting that it acts at an early step in homologous recombination to inhibit inappropriate recombination events. RECQL5 can also disrupt the RAD51-ssDNA filament, suggesting overlapping functions of these two RecQ helicases at an early step in homologous recombination.

RECQL5 interacts directly with MRE11 and NBS1 and partially inhibits the exonuclease activity of the MRE11/RAD50/NBS1 complex. RECQL5 also interacts specifically with hyperphosphorylated RNA polymerase II, but a role for this interaction of RECQL5 with RNA polymerase II has not yet been confirmed.

RECQL forms lower and higher order oligomers, which are differentially active in DNA unwinding and ssDNA annealing, respectively.

One of the central unanswered questions about RecQ helicases is to what extent is the function of each of the five mammalian RecQ helicases unique and non-redundant, and to what extent do their functions overlap? Unicellular model genetic organisms such as *Escherichia coli*, *Saccharomyces cerevisiae* (budding yeast), and *Schizosaccharomyces pombe* (fission yeast) express a single RecQ helicase; *Drosophila melanogaster* expresses three RecQ helicase genes, *Blm*, *RecQL4*, and *RecQL5*, and a gene that shares homology with the N-terminal exonuclease region of *WRN*; and *Caenorhabditis elegans* has four RecQ family members, *RecQL*, *BLM*, *WRN*, and *RecQL5*. In light of this variation, a related question is how did the RecQ gene family evolve and what evolutionary forces generated the RecQ families of vertebrate genomes that encode five RecQ helicases. Importantly, the clinical and cellular phenotypes associated with mutations in human *BLM*, *WRN*, and *RECQL4* are highly distinct, indicating that human *BLM*, *WRN*, and *RECQL4*, each have at least some unique, non-redundant biological functions. Nevertheless, the biological functions of *BLM*, *WRN*, and *RECQL4* might still overlap. Very recent biochemical studies show that each RecQ helicase exists *in vivo* in large, highly specific multisubunit protein complexes and supercomplexes, supporting the idea that each RecQ helicase has unique functions. Many presentations at this workshop highlighted advances in defining and characterizing RecQ-containing protein complexes and in identifying the distinct biological functions of BLM, WRN, and RECQL5. Although the roles of RECQL and RECQL4 are less well understood than those of BLM, WRN, and RECQL5, significant new results concerning the biological, biochemical, and genetic functions of these two proteins were also reported at this workshop.

One of the hallmarks of BS is genomic instability, characterized by excessive chromosome breakage and increased exchange between homologous sequences, as exemplified by increased sister-chromatid exchange (SCE) in proliferating mitotic cells. BS cells exhibit a hyper-recombination phenotype that is unique among human genetic disorders. Biochemical studies have shown that BLM, in combination with the breaking and rejoining enzyme Topoisomerase III (TopoIII α), can dissolve double Holliday junction structures *in vitro*, an activity that other human RecQ helicases do

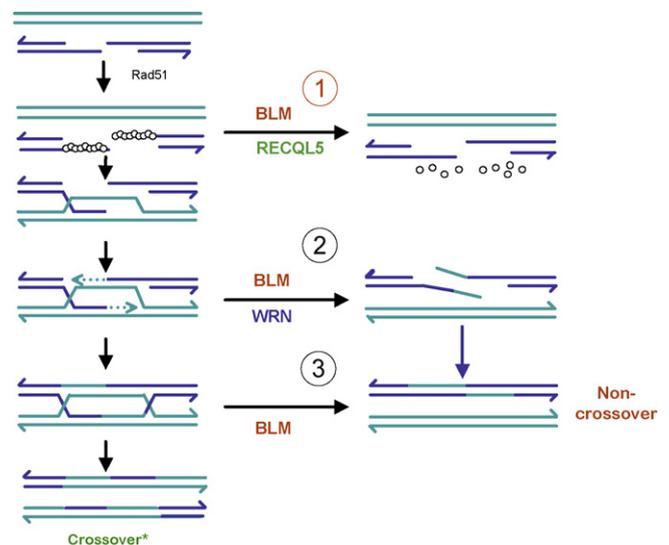


Fig. 1. Proposed pro- and anti-recombinogenic roles for BLM, WRN, and RECQL5. In step 1, BLM and RECQL5 disrupt the RAD51-ssDNA filament. In steps 2 and 3, BLM and WRN promote non-crossover resolution of recombination intermediates, including the double Holliday intermediate. BLM is the only RecQ helicase capable of promoting dissolution of a double Holliday junction. *Only one of the two possible crossover products is shown.

not possess. Although RecQ helicases play roles in many aspects of DNA metabolism, much research is focused on understanding the roles of these enzymes in repairing DNA double-strand breaks (DSBs). Evidence presented at this workshop suggests that during DSB repair, BLM increases genetic stability by promoting dissolution of recombination intermediates in a manner that favors non-crossover products (Fig. 1; steps 2 and 3). *In vitro*, BLM can destabilize the RAD51 single-stranded DNA (ssDNA) nucleoprotein filament (Fig. 1; step 1), a function that might increase genome integrity by protecting stalled replication forks from unnecessary recombination events. Consistent with a role at stressed replication forks, data presented at this workshop also demonstrate that, in BLM-deficient cells, replication fork progression is slower and inter-origin distance is smaller, even in the absence of induced DNA damage. Defects in BLM but not WRN reduce the efficiency of replication re-start in cells with methylmethane sulfonate (MMS)- or hydroxyurea (HU)-induced DNA damage, and both BLM and WRN play roles in early stages of activation of the S-phase DNA damage checkpoint.

WRN is unique among RecQ helicases in possessing 3' to 5' DNA exonuclease activity. The combined action of WRN helicase and exonuclease may bias DSB repair toward non-crossover products, thus increasing genetic stability, by promoting flux through the strand displacement ssDNA annealing (SDSA) branch of the DSB repair pathway (Fig. 1; step 2). Although both BLM and WRN interact with the telomere-specific protein TRF2, the cellular phenotypes of WRN-deficient cells clearly implicate WRN in the maintenance and replication of telomere DNA. WRN may also play critical roles in base excision repair and in the response to interstrand cross-links (ICLs).

Recent biochemical and genetic studies suggest that RECQL5 is likely to play a similar yet distinct biological role from BLM in mouse and human cells. The phenotype of a *Recql5* knockout mouse includes cancer susceptibility, and the cellular phenotype of *Recql5*-deficient mouse embryonic stem (ES) cells includes increased SCE. *Recql5*-deficient but not *Blm*-deficient mouse ES cells show increased chromosomal aberrations in response to camptothecin (CPT)-induced DNA damage and hypersensitivity to cell killing by CPT. However, like BLM, RECQL5 promotes dissolution of the RAD51-ssDNA filament *in vitro* (Fig. 1; step 1). RECQL5 is also likely to play roles both early and late in the response to DNA damage *in vivo*.

The subcellular localization of RECQL4 is regulated by acetylation, such that acetylated RECQL4 is sequestered in the cytoplasm, where it may play an important as yet unknown biological function. Recent immunofluorescence studies suggest that RECQL4 is recruited rapidly to DNA damage-induced DSBs in mitotic cells, and that this recruitment may be independent of WRN and BLM.

These and other results presented at this workshop are summarized in Box 1 and are described in detail below.

3. Connecting research scientists and clinicians with persons with BS and their families

On the evening of the first day of this workshop, a special joint session was held with Bloom's Connect. This session included presentations by Sheryl Grossman (Bloom's Connect founder), James German (Weill Medical College of Cornell University), Maureen Sanz (Molloy College), and Richard Gladstein (The Bloom's Syndrome Foundation; <http://www.milogladsteinfoundation.org>).

Sheryl Grossman founded Bloom's Connect in 1996, as a forum for communication for persons with BS and their families. At the workshop, Grossman described her experience as a person with BS

Box 2. Report from the Bloom's Syndrome Registry

BS registered population	N	%
Persons	264	–
Families	218	–
Families with 1 BS member	178	–
Families with 2 BS members	36	–
Families with ≥3 BS members	4	–
BLM mutations		
Unique mutations in BLM	45	–
Recurring mutations in BLM	19	–
Major medical complications		
Chronic lung disease	7	2.7
Diabetes	43	16.3
Cancer (# individuals)	118	44.7
Cancer (all cancers all sites)	177	–
Hematopoietic	20	–
Lymphoid	44	–
Epithelial	93	–
Other	20	–

and offered to interact with researchers to enhance their understanding of what is like to live with BS.

James German, a pioneer in clinical research on BS, discovered and characterized many aspects of the cellular phenotype associated with BS, in particular, the unique chromosomal instability that is exhibited in BS cells. German presented the outline of the book he is writing on BS. The book is entitled "The Bloom's Syndrome Story: The Business of Finding Out."

Maureen Sanz presented a report from the Bloom's Syndrome Registry, which tracks clinical statistics on persons with BS worldwide. Data presented by Sanz are summarized in Box 2.

Richard Gladstein founded The Bloom's Syndrome Foundation in 2004, shortly after his son Milo was diagnosed with BS. Gladstein described the experience of being the parent of a BS child. The Bloom's Syndrome Foundation funds research on BS and closely related research topics.

During this session and throughout this workshop, persons with BS and their families had the opportunity to interact with BS researchers and clinicians (Fig. 2). In addition, persons with BS and their families had the rare chance to interact with each other. There was consensus among all participants that these interactions were an important and valuable aspect of the workshop. Remarks from some of the non-scientist participants are included in Box 3. Persons with BS and their families expressed hope for future improvements in the quality of their lives, and provided encouragement and motivation to researchers in their quest for therapeutic approaches to BS and related diseases.

4. Workshop presentations

4.1. Session 1: Biochemistry and genetics of RecQ helicases

Patrick Sung (Yale University)

BLM/TopoIII α /BLAP-dependent Holliday junction processing

The mechanism by which BLM is regulated in human cells remains poorly understood. BLM copurifies and forms a complex with at least three polypeptides. These polypeptides include TopoIII α and the BLM-associated proteins BLAP75 and BLAP18. While TopoIII α plays an important catalytic function in the BLM complex, BLAP75 appears to play a structural and possibly regulatory role. BLAP75 is evolutionarily conserved and has an N-terminal oligonucleotide binding domain (OB-fold) that, contrary to expectation, mediates its binding to BLM and TopoIII α . Deletion of the OB-fold inactivates binding of BLAP75 to BLM and TopoIII α , whereas deletion of the C-terminal region of BLAP75 disrupts the DNA binding activity of BLAP75. Purified BLAP75



Fig. 2. Photos of Workshop participants: (Upper) Participants of the scientific workshop (left to right) James German, Vilhelm Bohr, Nathan Ellis, and Richard Gladstein with Stacey Dentz (front). Photographed by Kelly Hoadley. (Lower) Participants of the support group meeting (left to right) Sheryl Grossman, Stacey Dentz, Melissa LaCouvee, and LaCouvee family members. Photographed by Karen Grossman. Senior Workshop Presenters.

strongly stimulates BLM/TopoIII α double Holliday junction dissolution and helicase activities *in vitro*. This stimulation is specific for BLM/TopoIII α (is not observed with WRN or *E. coli* Top3), and it does not require the C-terminal DNA binding domain of BLAP75. The specificity of the interaction between BLAP75 and BLM/TopoIII α is further demonstrated by the fact that a single point mutation, K166A, inactivates the physical interaction between BLAP75 and TopoIII α and destroys the ability of BLAP75 to stimulate the double Holliday junction dissolution activity of the BLM/TopoIII α complex.

Robert Bambara (University of Rochester)

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

BLM, ssDNA binding protein RPA, and the flap endonuclease FEN1 play putative roles in lagging strand DNA replication in human cells. *In vitro* studies suggest that RPA can melt duplex regions of structured ssDNA and promote subsequent ectopic binding and annealing of homologous ssDNA fragments. If such reactions occur *in vivo*, RPA could potentially promote promiscuous binding of ssDNA at ectopic genomic sites, which might then lead to undesirable HR-mediated outcomes. A model *in vitro* system was used to examine whether BLM and FEN1 interact with and/or process the proposed undesirable recombination intermediate that might be formed during lagging-strand DNA synthesis. The results show that BLM strand exchange activity or BLM helicase coupled with FEN1 cleavage activity can release the invaded ssDNA from the model DNA substrate. Interestingly, in this *in vitro* system, BLM helicase disrupts recombination intermediates, even in the presence of RPA, supporting the idea that BLM plays an anti-recombinogenic role during lagging-strand synthesis *in vivo*.

James Keck (University of Wisconsin)

Structural and cellular mechanisms of bacterial RecQ DNA helicases

E. coli RecQ is the prototypical member of the RecQ helicase family. The N-terminal catalytic core region of RecQ includes the helicase and RecQ-Ct domains, the latter of which is comprised of cysteine-rich helical and winged-helix subdomains. The C-terminal region of RecQ includes the HRDC (Helicase and RNase D C-terminal) domain. Winged helix domains typically bind

duplex DNA and the helicase subdomain of RecQ probably binds ssDNA, suggesting that these two domains mediate double-stranded DNA (dsDNA) and ssDNA binding, respectively, during the helicase reaction. Winged helix motifs also mediate protein-protein interactions. For example, the winged helix subdomain of BLM mediates interaction with RPA, suggesting that *E. coli* SSB might interact with the winged helix subdomain of RecQ. In fact, SSB copurified with TAP-tagged RecQ, indicating functional conservation of this interaction from bacteria to human cells. Binding of SSB to RecQ is mediated by the C-terminal 9 amino acids of SSB and SSB stimulates the helicase activity of RecQ. Mutation in RecQ residues R503 or R425 eliminates binding of SSB to RecQ, inhibits RecQ helicase activity, and reduces the ability of SSB to stimulate RecQ helicase activity. Thus, RecQ R503 and R425 are critical for the SSB/RecQ interaction.

4.2. Session 2: RecQ helicase functions in recombination

Douglas Bishop (University of Chicago)

The energetics of homologous recombination reactions

DNA strand exchange is energetically neutral and does not require ATP hydrolysis. However, strand exchange proteins (the recombinases) are typically ATP-dependent and catalyze ATP-hydrolysis during *in vitro* strand exchange. One possible explanation for this apparent contradiction is that recombinases bind the strand exchange reaction product, and ATP hydrolysis is required to release them from the reaction product so that new reaction cycles can be initiated. This idea is consistent with a significant amount of *in vitro* and *in vivo* experimental data. For example, in yeast, Tid1, an ATP-dependent DNA translocase (or its paralog Rad54) is required to release the meiotic recombinase Dmc1 from undamaged DNA in $\Delta spo11$ strains. In addition, overexpression of the mitotic recombinase Rad51 is toxic in *tid1* mutant strains but has no effect in wild type strains. The toxicity of Rad51 overexpression in *tid1* mutant strains is associated with the appearance of ssDNA-independent Rad51 foci that persist throughout the cell cycle. This observation suggests that the function of Tid1 is to prevent product inhibition of Rad51. In the absence of Tid1, Rad51 accumulates in dsDNA-associated foci, and this accumulation has the effect of reducing the pool of available Rad51 for DSB repair, which in turn reduces cell viability. These

Box 3. Thoughts of some of the participants of the Bloom's Syndrome Support Group**Jeff Sopher, Maryland, USA**

The Bloom's Connect conference set up by Sheryl Grossman has meant a lot to me. It was truly amazing to meet others with Bloom's syndrome and to meet parents of children with Bloom's.

Life gets pretty tough for a child that grows up different than everyone else. I know my daughter will face many of the same challenges that others with Bloom's syndrome do. As a father, my job is to protect my children. That job is hard enough when everything is perfect. It is even harder when they are not, especially when there is nothing I can do about it other than just to be there for her. A great stress was lifted from me when I had the opportunity to speak to people with Bloom's syndrome who are in their 20's and 30's. As my daughter continues to have medical issues, as a parent I start to think about the potential for a shortened life. That is very hard to deal with. You don't know what it means just to meet and talk to people with Bloom's syndrome that are my age and still healthy.

From the medical aspect of the conference, I truly feel that all of us being able to be face to face with the doctors and scientists gave them a great realization that people with Bloom's syndrome are not just DNA strands. They are people, many little children, and they all have parents and families that want this syndrome to be fixed. I am grateful for that opportunity.

I want Bloom's Connect to continue its efforts in making knowledge of Bloom's syndrome more widespread. The information we, as parents, have gained from Bloom's Connect has been invaluable.

Melissa LaCouvee, Quebec, Canada

Thoughts about the Bloom's Connect conference: I was really nervous to meet other people with Bloom's, I knew there were other people who had it but seeing them made it a reality. I'm so glad I went. It was totally worth it. I made new friends and learned that if other people with Bloom's can lead a normal life so can I. I hope that we will be able to keep in touch.

Henri van den Hurk, The Netherlands

About 8½ years ago, our son Toon was born. Although very small, he was a sparkling and happy boy. We were very proud. He developed well and after a few months the summer season started in The Netherlands. With the first sun, he got a red rash on his face and some spots. We went to the local doctor, and he gave us some ointment but this did not help. We went to the local hospital and afterwards to an academic hospital in Nijmegen. There, the dermatologist also could not figure out what the cause was. He asked the help of a specialized doctor for children from the department of Immunodeficiency (Dr. Corrie Weemaes) and they wanted to test for Bloom's syndrome. This test was right on the nose.

We were not aware of any research on Bloom's syndrome. Sometimes we read a recent article on the internet, but this was very specialized (and therefore hard for us to understand). But, as far as we knew, there was no cure and nobody was working on it. Then, we read about a film producer and his wife, Richard and Lauri Gladstein, who were raising funds on behalf of their son Milo. We found out that there was research going on and that there were researchers alive who were interested in the syndrome. We therefore also started a fund-raising (www.bloomssyndrome.eu) in the hope that every dollar would help.

We also learned that a conference on Bloom's syndrome would be organized in May 2008. We asked Toon if he wanted to go to the USA. Of course he wanted to know more about Bloom's syndrome, but maybe more than that he wanted to be

on top of a skyscraper (he is an 8-year old boy, does not speak English and the highest building in The Netherlands is a windmill).

So Anne-Marie and Toon went to the Bloom's Connect Meeting and I went to the Science Meeting. With regards to the former, we wanted to meet others with Bloom's syndrome and exchange experiences, and Toon wanted to meet other people with Bloom's syndrome, too. This peek in the future caught him with surprise. His main concern at the moment is how tall he will be when he is older. "How will others see that I am older than my brothers if they are taller than me?" All in all, it felt very good to meet and talk to people in the same situation and make new friends. It was a great success, well done!

As for the Science Meeting, I knew that I probably would not understand much. I learned that a fork was not something you eat with and that my thoughts about a Holliday Junction were different from the rest. To be honest, I understood just a little bit more than the words "welcome" and "the end." But this did not matter to me: I saw about 70 people, scientists and researchers together in a room talking about Bloom's for two days. Big names and Big institutions. Being the father of a young boy with Bloom's syndrome, I hope you can imagine we are interested in a cure, a solution for this syndrome. I understand that "we" are in the phase of understanding how it works and finding out what exactly goes wrong with the DNA. But I also know that you have to take this first step before you can make a second one. I have a strong hope, a strong belief that the second step will be taken soon. I am sure that a lot of people, not only those with Bloom's, will benefit from this. I also hope, in due time, that Toon will be able to thank you in person for your work.

results are consistent with recent studies of *E. coli* recombinase RecA. RecA normally binds ssDNA with higher affinity than dsDNA. The RecA mutant E96D produces ATPase-deficient protein that binds ssDNA and dsDNA with equal affinity, which is toxic to the cell. RecA-E96D promotes strand exchange *in vitro* and recombination *in vivo*. However, in novel cell biological studies, RecA-E96D was shown to be tightly associated with the *E. coli* nucleoid in the absence of DNA damage, whereas wild type RecA is not. Thus, the toxicity of RecA-E96D in *E. coli* may be analogous to the toxicity of Rad51 overexpression in *tid1*-deficient yeast.

Alexander Mazin (Drexel University)

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

The cellular phenotype of *BLM* mutants is characterized by hyper-recombination, suggesting that *BLM* normally plays an anti-recombinogenic role in mammalian cells. The activities of *BLM* on putative recombination intermediates has been studied extensively; however, the possibility that *BLM* interacts with the RAD51-ssDNA filament to inhibit HR at an early stage has not been examined closely. *In vitro* studies show that *BLM* inhibits D-loop formation by RAD51 and that *BLM* promotes dissociation of the RAD51-ssDNA filament in an ATP-dependent manner. A similar process may occur *in vivo*, since it has been reported that overexpression of *BLM* in HU-treated cells inhibits formation of RAD51-staining foci and promotes a diffuse distribution of RAD51 in the nucleus. *BLM* helicase can also stimulate DNA polymerase η -dependent strand displacement DNA synthesis *in vitro*. This biochemical function of *BLM* may support error-free lesion bypass repair during DNA replication in human cells.

Yilun Liu (Yale University)

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

RAD51 paralogs are thought to regulate the availability of recombinase to its substrate, but they have other functions. Human cells express five RAD51 paralogs, RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3. These proteins form two distinct multisubunit complexes, which can be resolved by gel filtration chromatography. The CX3 complex, which includes RAD51C and XRCC3, copurifies with a Holliday junction resolution activity. The BCDX2 complex includes RAD51B, RAD51C, RAD51D, and XRCC2. Interestingly, CX3 and RuvA, a protein involved in resolution of Holliday junctions in *E. coli*, inhibit RECQL5-catalyzed branch migration of Holliday junctions, while BCDX2 does not. This observation suggests that CX3 and RECQL5, and possibly other RecQ helicases, may play antagonistic roles during the response to DNA damage or replicative stress. Mass spectrometry studies show that the protein-interacting partners of each of the five human RecQ helicases are highly distinct groups of proteins, which supports the idea that each RecQ helicase in human cells plays a unique biological role. The protein-interacting partners of RECQL5 include RNA polymerase II as well as several transcription elongation factors. A 9 amino acid motif near the center of RECQL5 is required for binding to dephosphorylated RNA polymerase II; however, the 9 amino acid motif as well as the C-terminal region of RECQL5 is required for binding to hyperphosphorylated RNA polymerase II. The biological significance of the putative interaction between RECQL5 and RNA polymerase II is not yet understood.

4.3. Session 3: Functions of the non-Bloom RecQ helicases

Alessandro Vindigni (International Centre for Genetic Engineering & Biotechnology, Trieste)

Structural and functional studies on the human RECQL helicase

RECQL was the first human RecQ helicase to be identified. Similar to phenotypes in BS cells, depletion of RECQL induces spontaneous γ -H2AX foci and more frequent SCE. However, because RECQL is constitutively expressed during the cell cycle, while expression of BLM is cell cycle dependent, it has been proposed that RECQL has a unique biological role that is not redundant with the role of BLM or other RecQ helicases. Supporting this claim, the DNA substrate specificities of RECQL are notably distinct from the DNA substrate specificities of BLM. For example, BLM unwinds G quartet (G4) DNA and RNA-DNA hybrid structures, whereas RECQL does not unwind these DNA substrates even in the presence of the RPA, which can stimulate its DNA helicase activity on its preferred DNA substrates. In addition, RECQL fails to promote regression of a model DNA replication fork and is very inefficient in displacing plasmid D-loops lacking a 3'-tail. In contrast, RECQL, but not BLM, is able to resolve immobile Holliday junction structures lacking an homologous core, even in the absence of RPA. RECQL, like other RecQ helicases, catalyzes both ssDNA annealing and dsDNA unwinding. Oligomeric state regulates the balance between these dual opposite enzymatic activities. Higher-order oligomers of RECQL catalyze DNA strand annealing, whereas lower-order oligomers (*i.e.*, monomers or dimers) catalyze DNA unwinding. Mutagenesis studies show that the N-terminal region (1–48) of RECQL is necessary for higher-order assembly state and confirms that smaller oligomers are involved in DNA unwinding. The N-terminal region is however necessary for the Holliday junction resolution activity of RECQL suggesting that this domain, or the formation of higher-order assembly states promoted by the N-terminus, is essential for the ability of the protein to unwind Holliday junctions. By examination of the *E. coli* crystal structure and comparison of amino acid homologies in the winged helix subdomain, RECQL contains a unique beta hairpin loop with a tyrosine residue at the tip. The analogous loop region in *E. coli* RecQ

and WRN is shorter and structurally distinct. Mutations in this loop inactivate the RECQL helicase activity, indicating that this hairpin plays a crucial role in DNA strand separation, as already suggested for other helicases in Superfamily 2. These data suggest that this hairpin loop region may be an important determinant of the biochemical and biological specificity of RECQL.

Pavel Janscak (University of Zurich)

Biochemistry and function of RECQL5 protein

Human cells express three isoforms of RECQL5. The largest RECQL5 isoform, RECQL5 β , is 991 amino acids long and has both ATP binding, ATPase and, DNA helicase activity. The shorter RECQL5 isoforms, RECQL5 α (410 aa) and RECQL5 γ (435 aa) bind ATP but lack ATPase and DNA helicase activity. RECQL5 β has a weak DNA helicase activity that is stimulated by RPA or SSB, and it has robust ATP-dependent ssDNA annealing activity. RECQL5 binds RAD51 and disrupts the RAD51-ssDNA filament. In undamaged cells, RECQL5 is diffusely distributed in the nucleus. In cells with laser-induced DSBs, RECQL5 is recruited to DSBs, as evidenced by its presence in γ -H2AX foci, and it colocalizes with the MRE11/RAD50/NBS1 (MRN) complex. Furthermore, RECQL5 physically interacts with MRE11 and NBS1, and it partially inhibits the exonuclease activity of the MRN complex. Recruitment of RECQL5 β to DSBs does not occur in MRE11-deficient cells, but it does occur in cells in which resection of DSBs by MRN is inhibited. These data indicate that RECQL5 interacts specifically with the MRN complex. Because RECQL5 inhibits MRN complex activity, the data suggest that RECQL5 plays an important role in regulation of DSB repair in human cells.

Igor Stagljär (University of Toronto)

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

Defects in *RECQL4* are associated with three human autosomal recessive syndromes: Rothmund-Thomson, RAPADILINO, and Baller-Gerold. Although the biochemical properties of RECQL4 suggest roles in DNA replication and DNA repair, a fraction of RECQL4, in contrast to the other RecQ helicases, localizes to the cytosol in undamaged cells. In overexpression studies in cultured human cells, RECQL4 and p300 (a protein acetyl transferase) were shown to interact, and RECQL4 was acetylated in a lysine-rich region from amino acids 376 to 386. Mutational studies indicate that any of the RECQL4 residues 376, 380, 382, 385, and 386, can be acetylated, and p300 can acetylate more than one of these five lysines on the same peptide. The RECQL5-p300 interaction is specific because it is not observed with other human protein acetyl transferases (pCAF, GCN5, HAT1). Purified p300 physically interacts with purified RECQL4 *in vitro* and acetylates it. Mutation of the lysine acetylation sites in RECQL4 resulted in sequestration of RECQL4 in the cytosol, suggesting that this region plays a role in nuclear localization of RECQL4. This idea was confirmed by showing that fusion of the putative nuclear localization signal of RECQL4 to β -galactosidase caused nuclear localization of β -galactosidase, whereas fusion of a mutant nuclear localization signal in which arginines were substituted for the lysines did not. Furthermore, co-overexpression of RECQL4 and p300 *in vivo* caused an increase in the proportion of cytosolic RECQL4, suggesting that acetylation of RECQL4 by p300 regulates its nuclear localization. In contrast, the proportion of cytosolic RECQL4 did not increase in cells treated with leptomycin, which is known to inhibit nuclear export. These data are consistent with the hypothesis that p300 regulates the subcellular localization of RECQL4 *in vivo* and that RECQL4 plays an as yet undetermined but biologically significant role in the cytosol in human cells.

4.4. Session 4: ELLISON FOUNDATION SESSION—RecQ functions in cancer and aging

Vilhelm Bohr (National Institute on Aging, NIH)

Functions of WRN and other RecQ helicases in DNA repair

RecQ helicases are thought to play cooperative but distinct roles in several DNA repair pathways including DSB repair, HR repair, base excision repair, telomere repair, and non-homologous end joining. The RecQ-Ct domain in WRN mediates significant functional interactions with multiple DNA repair proteins, including the breast cancer protein BRCA1, FEN1, and DNA polymerase β . Oxidative DNA lesions accumulate in WRN-defective cells, and WS cells are hypersensitive to psoralen-induced ICLs. Recent studies indicate that WRN colocalizes with ICLs and with proteins involved in ICL repair. These data suggest that WRN plays a specific role in base excision repair and ICL repair in human cells. WRN may also play a role upstream of ATR in the response to ICLs and promote activation of an S-phase DNA damage-induced checkpoint.

RECQL4 may also play a distinct role in the response to ssDNA and dsDNA strand breaks in human cells. Recent immunohistochemical studies suggest that RECQL4 co-localizes with γ -H2AX and the p53 binding protein 53BP1 in irradiated fibroblasts and that repair foci persist longer in RECQL4-deficient cells than in wild type cells. RECQL4 is recruited to laser-induced DNA lesions rapidly (<30 min) and dissociates from these DNA lesions before WRN or BLM. RECQL4 is recruited more efficiently to putative DSBs breaks in cells treated with high-dose laser irradiation. Recruitment of RECQL4 to these lesions is observed in cells deficient in WRN, BLM, or ATM, suggesting that RECQL4 acts independently of WRN and BLM at sites of laser-induced DNA lesions. Truncation of RECQL4 eliminates the ability of RECQL4 to bind to DNA laser-induced DNA lesions *in vivo*. Additional studies are required to define the role of RECQL4 in repair of ssDNA and dsDNA breaks *in vivo*.

Patricia Opresko (University of Pittsburgh)

RecQ helicases' functions at telomeres

A significant amount of evidence suggests that WRN and BLM play specific roles in telomere maintenance in human cells. For example, WRN and BLM localize to telomeres during S-phase in telomerase-deficient cells and defects in WRN lead to telomere loss in fibroblasts. In addition, telomeric proteins POT1 and TRF2 interact with WRN and BLM, and POT1 strongly stimulates BLM- and WRN-mediated unwinding of several DNA substrates *in vitro*. However, stimulation of WRN helicase by POT1 only occurs if the DNA substrate carries the TTAGGG POT1 DNA recognition sequence. POT1 does not stimulate the helicase activities of *E. coli* RecQ or UvrD, suggesting that the interaction between WRN and POT1 is direct and species specific. Both POT1 and RPA stimulate unwinding of a telomeric D-loop DNA substrate (containing 10 telomeric repeats in the D-loop region) by WRN or by exonuclease-deficient WRN, but they do not directly alter the WRN exonuclease activity in the absence of helicase activity on telomeric DNA D-loop or fork substrates. Because POT1 and RPA promote release of the nearly full-length invading strand of the D-loop, it is possible that POT1 and RPA increase the rate or efficiency of WRN-catalyzed DNA unwinding. However, RPA is more effective than POT1 in stimulating unwinding of a telomeric D-loop by exonuclease-deficient WRN. These data are consistent with the possibility that WRN and POT1 cooperate to resolve replication or recombination intermediates in telomeric regions of human chromosomes.

Ray Monnat (University of Washington)

The Werner syndrome protein WRN as a fork 'spork'

WRN alleles in persons with WS are exclusively null alleles that inactivate both WRN helicase and WRN exonuclease. WS cells

carrying WRN null alleles have been characterized, but the biological and cellular impact of missense alleles of WRN have not been studied. This question was addressed using an *in vivo* assay that scores mitotic recombination events that convert G418 sensitive cells to G418 resistant cells. In this assay, WRN-deficient cells display a 25-fold reduction in rate of mitotic gene conversion from G418 sensitivity to G418 resistance. While exonuclease-defective or helicase-defective WRN alleles rescue cell viability in cells treated with cis-platinum, only wild type WRN rescues the mitotic recombination defect in WS cells. Mitotic gene conversion from LacZ⁻ to LacZ⁺ was also scored in wild type and WRN-deficient cells; this assay measures recombinant product in the absence of cell proliferation. The results of this experiment indicate that WRN-deficient cells initiate mitotic recombination, but fail to resolve recombination intermediates and fail to generate viable recombinant daughter cells. Thus, WRN deficiency can be considered a recombination deficiency syndrome. The effect of WRN deficiency on progression of DNA replication forks can be examined by "replication track" analysis, in which replicating cells are sequentially pulse-labeled with chloro-deoxyuracil and iodo-deoxyuracil, and chromatin spreads are visualized immunohistochemically using antibodies to these two nucleotide analogs. Track length can be measured in wild type or mutant cells treated with or without MMS or HU. The results of these experiments suggest that treatment with MMS slows replication fork progression significantly in wild type cells and fork movement is even slower after MMS treatment of WRN-deficient cells. Furthermore, defects in BLM but not WRN reduce the efficiency of replication re-start in cells treated with MMS or HU. These data strongly support the hypothesis that WRN and BLM play different roles in the response to replication stress in MMS- and HU-treated cells.

4.5. Session 5: RecQ helicases and cellular physiology

Nancy Maizels (University of Washington)

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

G-rich DNA sequences form unusual structures, called G4 DNA, in which the repeating unit is the G-quartet, a planar array of four guanines. G4 DNA forms spontaneously in regions bearing the signature motif G₃N_xG₃N_xG₃N_xG₃. G4 DNA is very stable once formed, and it can impede transcription and replication if not unwound. Several human DNA helicases unwind G4 DNA efficiently. These include RecQ family members BLM and WRN and the DEAH helicase FANCF. Genomic instability leading to cancer characterizes human disease in which any of these helicases is lacking. Bioinformatic tools can be used to estimate the G4 DNA-forming potential (G4P) of specific DNA sequences or genomic regions. We developed one such tool, G4P calculator, available on our laboratory website, and used it to evaluate G4P of all human genes and their upstream regulatory regions. Low or high G4P is shared by introns and exons, and is a property of genes, not local chromosomal environment. Genes with low and high overall G4P were functionally distinct: notably, tumor suppressor genes have much lower G4P and proto-oncogenes much higher G4P than the genomic average. Thus, human genes are under selection for G4P. This suggests that low G4P may be selected to ensure genomic stability. Human promoters are G-rich within 1 kb upstream and downstream of the transcription start sites, but essentially all G-richness upstream is due to CpG islands or canonical motifs for duplex DNA binding proteins, like SP1. Downstream of the transcription start sites, a significant minority of human genes contain a G-rich element on the non-template strand, at the very 5' end of intron 1. These G-rich intron 1 elements (GrIn1 elements) are not eliminated by masking CpG motifs or motifs for RNA

binding factors, and they are conserved through frogs. Their position at the very 5′-end of intron 1 would enable them to regulate events at the promoter, without affecting the structure of the mRNA.

Robert Brosh (National Institute on Aging, NIH)

Mechanisms of RecQ-like helicases in cellular DNA metabolism

RECQL is the smallest of the five human RecQ helicases. Biochemical analyses indicate that RECQL catalyzes DNA unwinding and ssDNA annealing. It is presumed that these complementary and opposing enzymatic reactions allow RECQL and other RecQ enzymes to mediate complex DNA transactions during recombinational DNA repair and recovery from replication stress. Consistent with this, RECQL has physical and functional interactions with RPA, RAD51, and a mismatch repair protein complex that includes MSH2, MSH6, MLH1, PMS2, and EXO1. A role for RECQL in DNA repair and the response to DNA damage is supported by the observation that RECQL becomes phosphorylated and relocates from the nucleolus to DNA damage-induced foci in cells treated with ionizing radiation. Furthermore, recent studies show that mouse embryo fibroblasts from *Recql* knockout mice and human cells in which *RECQL* is knocked down by siRNA are moderately hypersensitive to ionizing radiation-induced DNA damage, have higher rates of SCEs, and exhibit abnormal mitoses compared to control cells. Thus, although *Recql* knockout mice are viable and appear normal, and no human disease phenotype has been associated with *RECQL* mutation, deficiency of *RECQL* appears to confer a cellular phenotype.

FANCD1 DNA helicase is encoded by one of the thirteen genes associated with the human disease syndrome Fanconi anemia. FANCD1 has amino acid sequence homology to the seven common DNA helicase motifs, but it also shares limited homology with RecQ DNA helicases outside of the helicase motifs of Superfamily 2 helicases. FANCD1 binds to Breast Cancer protein 1 (BRCA1) and missense FANCD1 alleles (P47A and M299I) as well as premature translation termination mutations of FANCD1 are associated with increased risk of breast cancer. The biochemical properties of FANCD1 are distinct from WRN and the other RecQ helicases. For example, FANCD1 is a 5′ to 3′ DNA helicase, whereas RecQ helicases are 3′ to 5′ DNA helicases. The FANCD1 DNA helicase activity strongly prefers DNA substrates with a 5′-protruding ssDNA tail, whereas RecQ helicases have a more relaxed DNA substrate specificity. FANCD1 DNA helicase unwinds short DNA duplex regions up to approximately 25 bp in the absence of RPA, but its processivity increases in the presence of RPA. FANCD1 colocalizes with RPA at sites of DNA damage in cells treated with MMS, ionizing radiation, or HU. *In vitro* DNA helicase assays with forked DNA substrates carrying DNA backbone modifications suggest that FANCD1 processivity through damaged DNA may be facilitated by cooperative interactions between multiple FANCD1 monomers upstream of the DNA damage.

Yves Pommier (National Cancer Institute, NIH)

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

BLM deficiency can be considered a “replication disease,” in which the cellular phenotype can be attributed partly or entirely to defects in replication or replication-associated processes. One piece of evidence supporting this hypothesis is the fact that *BLM*-deficient cells undergo spontaneous checkpoint activation, forming significantly more γ -H2AX-, phospho-T68-Chk2-, and phospho-S1981-ATR-staining foci per cell than in normal undamaged cells. Direct evidence was sought using molecular combing, a technique that tracks the polarity and rate of progression of replication forks in single DNA fibers. These data demonstrated that DNA replication fork progression is significantly slower in *BLM*-deficient than in *BLM*-

proficient cells and that replication fork movement tends to be asymmetrical in *BLM*-deficient cells, whereas it is symmetrical in *BLM*-proficient cells. Interorigin distance is also shorter in *BLM*-deficient cells, which is likely to be a mechanism to compensate for slow replication fork progression in the absence of *BLM*. In *BLM*-deficient cells exposed to aphidicolin, which induces replicative stress, replication-associated DSBs as determined by γ -H2AX-staining and MUS81-staining foci are dramatically higher in number than in normal cells. In normal but not in *ATR*-defective cells, aphidicolin-induced DSBs are associated with phospho-T99-*BLM*. These data suggest that *BLM*, *MUS81*, and *ATR* cooperate in the response to replication stress, promoting formation of DNA damage-associated foci that allow resumption of DNA replication fork progression. *BLM*-deficient cells are also defective in the response to CPT, an anti-cancer agent that stimulates formation of topoisomerase 1-cleavage complexes in DNA. In particular, although greater loss of viability and higher than normal levels of topoisomerase 1 cleavage complexes are detected in *BLM*-defective compared to normal CPT-treated cells, formation of γ -H2AX-foci is delayed, and formation of TopoIII α -PML-staining foci is reduced in CPT-treated *BLM*-defective cells. Interestingly, in CPT-treated normal cells exposed to aphidicolin, phosphorylation of *BLM* on T99, which is mediated by *ATM* and *ATR*, fails to occur. These data support the hypothesis that *BLM* plays a significant role and cooperates with *ATM*, *ATR*, and TopoIII α in the response to replication stress and DNA damage in normal cells.

4.6. Session 6: Models of RecQ helicase deficiency

Joanna Groden (University of Cincinnati)

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

Persons with BS, who carry two defective alleles of *BLM*, have higher than normal rates of cancer, and cancers develop at earlier times than normal. In addition, the tissue distribution of cancer in BS and normal individuals is not identical, with persons with BS having a significantly higher risk of developing colorectal than other cancers. The effect of heterozygosity for *BLM* in humans on colorectal cancer risk has been reported to be elevated approximately 2-fold; however, several smaller studies have disputed this claim. Recent studies using mouse models for lymphoma, colorectal, and lung cancer suggest that in the mouse, haploinsufficiency for *Blm* (i.e., animals carrying one wild type and one knockout *Blm* allele) increases the risk of some but not all types of cancer. For example, the mean age of death for wild-type and *Blm* heterozygous mice injected with murine leukemia virus was 164.1 and 121.1 days, respectively, indicating a significant increase in susceptibility to lymphoma-induced death in *Blm*-heterozygous animals. Similarly, both tumor multiplicity and tumor progression/invasiveness was markedly higher in *Blm*^{-/+}, *Apc*^{min/+} doubly heterozygous mice compared to *Blm*^{+/+}, *Apc*^{min/+} mice. In contrast, heterozygosity for *BLM* had no detectable effect on initiation, progression, or regression of benign lung tumors induced by tissue-specific DOX-regulated expression of the *Fgf-10* oncogene in mice. Interestingly, the *BLM* locus is not susceptible to loss of heterozygosity in mouse models of colorectal or lung cancer, but evidence of higher than expected rates of gene conversion near *APC* were observed in *Blm*^{-/+}, *Apc*^{min/+} doubly heterozygous mice. These data suggest that tissue-specific modifiers of *Blm* modulate its function in normal and malignant, or pre-malignant, mouse cells, and that heterozygosity for *Blm* leads to higher than normal rates of recombination and/or mutation in the mouse. Additional experiments are needed to determine the implication of these results for cancer susceptibility in heterozygous carriers of *BLM*-defective alleles in humans.

Guangbin Luo (Case Western Reserve University)

Interrogating the molecular mechanisms of RecQ helicase functions using knockout models

The biological role of *Recq15* has been examined in a knockout mouse model generated by site-specific gene targeting. *Recq15*^{-/-} ES cells have a higher frequency of SCE than wild type cells and *Recq15*^{-/-} mice are highly prone to lethal sporadic cancer. Lymphoma and lung tumors were the most common sporadic cancers in *Recq15*^{-/-} mice. *Recq15*^{-/-} mouse ES cells are deficient in the response to DNA damage. For example, more γ -H2AX foci accumulate and persist in *Recq15*^{-/-} mouse embryo fibroblasts treated with CPT than in wild type CPT-treated cells, and these foci are associated with Rad51. These results suggest that defects in *Recq15* confer a hyper-recombination phenotype, which is consistent with the observation that *Recq15*^{-/-} cells carry out recombination-mediated repair of Scel-induced DSBs in a chromosomally located reporter gene much more efficiently than wild type cells. Interestingly, the rate of loss of heterozygosity is not higher in *Recq15*^{-/-} than in wild type cells, and the rate of CPT-induced chromosomal aberrations is higher in *Recq15*^{-/-} but not in *Blm*^{-/-} cells. Nevertheless, *Recq15*^{-/-} and *Blm*^{-/-} cells have a very similar SCE phenotype. *Recq15*^{-/-} cells are also hypersensitive to CPT, whereas *Blm*^{-/-} cells are not. These data suggest that *Blm* and *Recq15* have distinct functions in the response to DNA damage in mice and that *Recq15* plays a unique role in repair of CPT-induced lesions generated during cancer chemotherapy. Furthermore, *Recq15* may have more than one role in response to replication stress. At an early step, *Recq15* may prevent collapse of a stalled replication fork; alternatively, acting at a collapsed fork, *Recq15* may suppress undesirable outcomes involving HR repair. Thus, in response to replication stress, it appears that *Blm* and *Recq15* have non-redundant roles. Both are required for maintaining the integrity of the mammalian genome and for suppressing cancer.

Shunichi Takeda (Kyoto University)

The reverse genetic study of the vertebrate Blm and Fbh1 DNA helicases using the chicken DT40 cell line and Medaka fish

The F box DNA helicase I (Fbh1) gene has an F box motif in its N-terminal region and six canonical helicase motifs in its C-terminal region. Fbh1 is conserved in chicken, humans, and *Schizosaccharomyces pombe*, but not in *Saccharomyces cerevisiae*. Although *S. pombe* mutants lacking Fbh1 grow poorly, *Fbh1*^{-/-} chicken DT40 cells are viable, demonstrate normal resistance to most DNA damaging agents, and form DNA damage-induced Rad51 foci. However, *Fbh1*^{-/-} DT40 cells have higher levels of spontaneous SCEs than wild type cells. *Fbh1 Rad54* double knockout cells (*Fbh1*^{-/-} *Rad54*^{-/-}) are hypersensitive to CPT-induced lethality and generate more CPT-induced chromosome aberrations than wild type or single knockout cells. *Fbh1 Blm* double knockout cells (*Fbh1*^{-/-} *Blm*^{-/-}) cells are also much more susceptible to CPT-induced lethality than single knockout cells, and the frequency of chromosome aberrations is much higher in UV-treated *Fbh1*^{-/-} or *Blm*^{-/-} single knockout cells and in *Fbh1*^{-/-} *Blm*^{-/-} double knockout cells than in wild type cells. These data suggest that Fbh1 processes DNA repair intermediates generated by Rad54 in chicken DT40 cells and that Fbh1 cooperates with Blm to promote replication re-start and suppress crossover recombination events in cells with DNA damage.

The Medaka fish has several advantages as an experimental animal model system, including small genome size, minimal gene duplication, availability of inbred strains, tolerance to wide range of temperature for growth, and tolerance of sperm to freezing and thawing. In addition, the fish are transparent, which allows visual inspection for the development of tumors. A saturation ethylnitrosourea mutagenesis screen was performed and a library of

mutant Medaka fish generated and cryopreserved for future studies. A *p53*^{-/-} null allele from this library has a shortened life span and high incidence of epithelial tumors. In contrast, *Blm*^{-/-} Medaka show normal development and no increase in cancer. However, sperm from *Blm*^{-/-} Medaka fail to fertilize wild type Medaka eggs *in vitro*, indicating that BLM-deficiency in Medaka is associated with defective spermatogenesis. Oogenesis appears to be normal in *Blm*^{-/-} Medaka. The precise role of BLM in meiotic homologous recombination in Medaka is not yet known.

Jeff Sekelsky (University of North Carolina, Chapel Hill)

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

Drosophila Blm is encoded by *Mus309*, a gene identified in screens for mutagen-sensitive flies. The phenotype of *Blm*-defective flies includes female sterility, elevated spontaneous mitotic crossovers, mutagen-sensitivity, defective DSB repair, and defective meiotic recombination. It has been proposed that the biological role of *Drosophila Blm* is to “disrupt” D-loops or “dissolve” double Holiday junction-like DNA repair or recombination intermediates, thereby promoting formation of non-crossover products. Although *Blm*-deficient flies are viable, *Blm*-deficiency results in late pupal lethality when combined with a defect in *Drosophila Mus81*, a structure-specific nuclease that is involved in the resolution of Holliday junctions. *Blm*-deficient or *Blm*-deficient *mus81*-deficient flies demonstrate high or very high levels of apoptosis in larval imaginal discs, respectively. An N-terminal truncation mutant of *Drosophila Blm* (*Blm*^{N2}) is incompetent for Blm-dependent DSB repair but is viable in combination with *Mus81*-deficiency. Furthermore, mutation in *Drosophila Rad51* partially rescues lethality and apoptosis in imaginal discs of *Blm*-deficient *Mus81*-deficient flies. These results suggest that a spontaneous DNA lesion that requires *Mus81* or *Blm* but not *Rad51* for repair accumulates in *Blm*-deficient flies, and that this lesion, if unrepaired, is converted by *Rad51* into a toxic lesion that induces apoptosis. *Blm*-deficiency is also lethal in combination with *Mus312*-deficiency. *Blm*-deficient *Mus312*-deficient flies die as early pupae, have severe defects in cell proliferation, and have high rates of chromosome breakage. *Blm*-deficient *Mus312*-deficient flies are not rescued by *Rad51*-deficiency or *Blm*^{N2}. The human homolog of *Drosophila Mus312* is BTBD12, a protein that interacts with XPF and that is likely to play a role in repair of ICLs. The requirement for *Blm* in *Mus312*-defective flies may indicate that *Drosophila Blm* plays a role in repair of ICLs in flies.

4.7. Session 7: RecQ helicases in genome integrity

Steve Brill (Rutgers University)

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

Yeast cells carrying null alleles of the yeast homolog of BLM, *SGS1*, are viable in a wild type background, but they are lethal in combination with defects in two yeast proteins, Slx5 and Slx8. Slx5 and Slx8 are ring-finger domain proteins that form an Slx5/Slx8 heterodimer, and the dimer has E2-ubiquitin ligase activity. Yeast cells deficient in Slx5 or Slx8 accumulate high levels of high molecular weight SUMO-conjugated proteins. Biochemical studies show that the Slx5/Slx8 dimer binds to and ubiquitinates polysumoylated proteins. Interestingly, ubiquitin is targeted to the SUMO moiety on a model substrate, sumoylated Siz2, but not to unsumoylated Siz2. Furthermore, Slx5/Slx8 preferentially modifies the terminal SUMO moiety in polysumoylated Siz2. The *in vivo* substrates of Slx5/Slx8 and its exact biological role have not yet been determined. However, defects in yeast Top1 partially suppress the lethality of *sgs1 slx5*, and previous studies indicate that Top1 is sumoylated in CPT-treated yeast and that Top1

interacts with the SUMO pathway in yeast cells with DNA damage. Thus, yeast Sgs1/Slx5/Slx8 may play a role in processing sumoylated proteins that accumulate at or near replication forks in yeast cells with DNA damage.

Qin Yang (Washington University)

RecQ helicases, telomere recombination and maintenance

Approximately 15% of transformed cells maintain stable telomeres by a telomerase-independent mechanism known as the Alternative Lengthening of Telomere (ALT) pathway. ALT is characterized by heterogeneous telomere length, high levels of telomere-targeted HR, and dense-staining nuclear bodies rich in PML protein (ALT-PML bodies). ALT-PML bodies contain linear telomeric DNA, DNA repair proteins including RAD51, RAD52, RPA, MRN, and the telomere-binding proteins TRF1 and TRF2. BLM interacts with TRF2 and POT1 and localizes to telomeres in ALT cells. Mice express two POT1 proteins, POT1a and POT1b, encoded by two genes. In contrast, human cells express five POT1 isoforms (POT1v1 to v5), produced by alternative splicing of mRNA from a single human *POT1* gene. Human cells carrying dominant-negative TRF2 (DN-TRF2) show high levels of anaphase bridges and chromosome aberrations. However, overexpression of human POT1v1 or POT1v5 suppresses this phenotype, whereas overexpression of POT1v2 and POT1v4 do not. Overexpression of POT1v1 or POT1v4 also causes telomere lengthening in cells expressing dominant negative TRF2. Knockdown of endogenous POT1v1 but not POT1v5 causes shortening of the telomeric 3'-overhang in HT1080 and HCT116 cells, but both POT1v1 and POT1v5 are required to prevent telomere shortening and replicative senescence in human MRC-5 cells in culture. This process requires wild type p53 in cells knocked down for POT1v5 but not in cells knocked down for POT1v1. In addition, POT1v5 is preferentially expressed in human mismatch repair-defective cell lines. These results suggest that human POT1 isoforms play distinct roles in telomere maintenance in human cells. Furthermore, the results are consistent with the hypothesis that BLM, WRN, MSH2, MSH6, TRF2, and POT1 play cooperative roles in telomere maintenance in ALT-PML bodies.

Weidong Wang (National Institute on Aging, NIH)

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

BLM protein is a component of several multiprotein complexes in human cells. One of these complexes includes BLM, RMI1 (BLAP75), RMI2 (BLAP18), and TopoIII α . RMI1 has a conserved OB-fold domain in the N-terminal portion of the protein, and orthologs of RMI1 have been identified in yeast, *C. elegans*, zebrafish, mouse, and human cells. Depletion of RMI1 or BLM in human cells causes the same cellular phenotype: increased frequency of SCEs in the absence of DNA damage. RMI1 also stimulates BLM/TopoIII α -mediated dissolution of double Holliday junction structures *in vitro*. Recent studies show that RMI1 colocalizes with and binds to RMI2, and that RMI1 and RMI2 form an OB-fold mediated heterodimer, which bridges the interaction between TopoIII α and BLM. This conclusion is strongly supported by the observation that RMI2-K121A, an RMI2 mutant that does not bind to BLM but does bind to RMI1 and TopoIII α , fails to suppress the high-SCE phenotype of RMI2-null cells.

BLM is also associated *in vivo* with a protein complex called BRAFT. BRAFT is a super-complex containing two subcomplexes and at least 20 components. One of the two BRAFT subcomplexes is the Fanconi Anemia (FA) Core Complex, which contains 12 polypeptides. The clinical features of BS and FA are similar, including developmental abnormalities and cancer susceptibility, and the cellular phenotype of BS and FA cells includes genomic

instability and hypersensitivity to ICLs. These data support the idea that BLM and FA proteins may participate in a common DNA damage response pathway, which is consistent with the observation that BLM interacts physically with the FA Core Complex.

4.8. Session 7: RecQ helicases and control of recombination suppression

Nathan Ellis (University of Chicago)

SUMO modification of BLM and the regulation of anti-recombination

In undamaged cells, BLM localizes in nuclear storage sites known as PML nuclear bodies. The main structural protein of these bodies, PML, contains both sites for SUMO modification and a SUMO interaction motif (SIM). PML sumoylation and the PML SIM are required to form PML nuclear bodies. Thus, the function of PML nuclear bodies is thought to be regulated by the SUMO pathway. Like PML, BLM has a SIM and can be preferentially sumoylated at four lysines (K317, K331, K344, and K347). The biological importance of BLM sumoylation was revealed by mutating two of the preferred BLM sumoylation sites (K317 and K331) and expressing normal BLM or SUMO-mutant BLM in BLM-deficient cells. Cells that expressed SUMO-mutant BLM accumulated large numbers of DNA damage-independent γ -H2AX foci as well as significantly more HU-induced γ -H2AX foci compared to cells that expressed normal BLM. Interestingly, untreated cells that expressed SUMO-mutant BLM had normal levels of SCEs, and HU treatment failed to induce additional SCEs. Furthermore, cells that expressed SUMO-mutant BLM were hypersensitive to HU- and etoposide-induced cell killing. These data suggest that cells that express SUMO-mutant BLM are deficient in processing of replication-associated DSBs by HR. One possible explanation for these observations is that sumoylation of BLM is required to release BLM from sites of DNA damage, thereby suppressing the anti-recombinogenic activity of BLM.

Hocine Mankouri (University of Oxford)

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

Sgs1 is the sole RecQ helicase in *Saccharomyces cerevisiae*. This makes yeast an excellent model system for studying, as well as potentially revealing novel therapeutic approaches, human BS. *sgs1* mutants are sensitive to MMS-induced DNA damage and accumulate aberrant "X-shaped" replication intermediates following exposure to MMS. These replication intermediates can be detected on 2-dimensional DNA gels of yeast DNA using a probe for a 5 kb locus containing the early-firing DNA replication origin *ARS305*. The X-shaped replication intermediates that persist in *sgs1* mutants are prevented by abolishing HR repair, suggesting that they are unprocessed HR repair intermediates. *CSM2*, *PSY3*, *SHU1*, and *SHU2* (collectively referred to as the 'SHU genes') form a single epistasis group that genetically interacts with *SGS1*. Mutations in any of the *SHU* genes significantly reduces the persistent MMS-induced X-structures in *sgs1* mutants. Bioinformatic studies suggest that the Shu gene products may be homologs of Rad51 paralogs, and it has therefore been proposed that the Shu gene products form a complex that assists, or regulates, HR repair in yeast. Therefore, mutation of human *SHU* homologs (*SWS1* and *RAD51D*) may represent a novel strategy to similarly suppress BS phenotypes. *ESC2* is a SUMO-like protein that is required for viability of *sgs1* mutants in the presence of MMS. Similar to *sgs1* mutants, *esc2* mutants also accumulate MMS-induced X-shaped DNA structures, and *sgs1 esc2* double mutants appear to undergo catastrophic mitosis following recovery from a transient exposure to MMS. Therefore, the human *ESC2* homolog, *NIP45*, may similarly be crucial for the viability of BS cells.

Ralph Scully (Harvard Medical School)

Control of sister-chromatid recombination in mammalian cells

A reporter system has been developed to facilitate molecular analysis of sister chromatid recombination (SCR) products in wild type and BLM-deficient human cells. This reporter system includes tandem GFP reporter genes, where the upstream GFP gene is truncated and the downstream GFP gene has an engineered I-Sce1-cleavage site. In addition, a re-arranged blastocidin resistance gene lies in between the two GFP reporter genes. This system allows the quantification of relative frequency of short- and long-track gene conversion events in cells of different genotypes. In wild type cells, most I-Sce1-induced SCR events are characterized by short gene conversion tracts. When long-tract gene conversion does occur in wild type cells, it is not usually associated with crossing over. A defect in RAD51C causes an ≈ 5 -fold increase in long gene conversion tracts. Attempts to establish this reporter gene system in BS cells revealed that BLM deficiency is associated with a high level of spontaneous SCR and reporter gene instability. Molecular analysis suggests that SCR events in BLM-defective cells are not associated with increased frequency of crossing over but are associated with long-tract gene conversion and concatamerization of repeated sequences in the reporter gene cassette.

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