FINAL REPORT
OTKA-NNF2 85613
Molecular mechanisms of genome maintenance
1/4/2011-31/3/2012

1. OVERALL GOAL OF THE PROJECT

The work performed in this project was a 1-year continuation of project NNF78783, entitled identically to the current work. The overall goal of the project was to elucidate the molecular mechanisms by which RecQ-family DNA helicases (RFHs) contribute to the maintenance of genome integrity. RFHs are present in organisms ranging from bacteria to humans. They exert various pro- and anti-recombinogenic activities to enable repair of DNA lesions and error-free transmission of genetic information during replicative processes. Loss of RFH function leads to severe damages to genome integrity, implying that the activities of these enzymes are essential for cells to avoid apoptosis and malignant transformation. In this project we investigated the action of *E. coli* RecQ and human Bloom's syndrome (BLM) and RecQ5β helicases.

2. SPECIFIC AIMS OF THE PROJECT

The Specific Aims of the reported project stemmed from the results of project NNF78783. After elucidating the DNA-restructuring molecular processes driven by BLM and RecQ in project NNF78783, we focused on the interplay between the molecular activities of helicases and other proteins playing key roles in homologous recombination (HR)-based repair processes.

Aim 1: Effect of human BLM and RecQ5β helicases on the formation and disassembly of Rad51 nucleoprotein filaments

The key step during the initiation of HR is nucleoprotein formation on the ssDNA strand by the Rad51 recombinase. In this Aim we set out to determine the linkage of Rad51 enzymatic steps to filament formation and disassembly. We also investigated the influence of BLM and RecQ5β helicases and fragments of the BRCA2 protein on Rad51 nucleoprotein formation and disassembly. These mechanisms were proposed to be crucial in the quality control of HR.

Aim 2: Cooperation between ssDNA binding proteins and RFHs during translocation along and unwinding of DNA strands

It was proposed that a well-defined physical interaction between single-stranded DNA binding (SSB) and RecQ proteins is crucial for their coordinated action leading to initiation of HR. In this Aim we investigated how SSB influences the enzymatic and DNA-restructuring activities of RecQ.

3. RESULTS ARISING FROM THE PROJECT

We have produced a large amount of experimental data related to each Specific Aim. We have published one research article during the project period, and two other articles are currently under review (see Section 4). In the published article we reported the surprising discovery that the winged helix domain of human BLM is not necessary for various mechanochemical activities of the enzyme, including the catalyzed disassembly of Rad51 nucleoprotein filaments. In one work under review, we determined the oligmeric state of BLM bound to various HR intermediates. Contrary to previous views, we found that the enzyme performs most HR activities in a monomeric form. In the other work under review, we report the precise mechanochemical parameters of the translocation of *E. coli* RecQ helicase along ssDNA.

Regarding the rest of the project, we are in process of analyzing the data, formulating conclusions and writing manuscripts. Below I provide representative examples of the unpublished results.

Aim 1: Effect of human BLM and RecQ5β helicases on the formation and disassembly of Rad51 nucleoprotein filaments

a) **Kinetics of human Rad51 filament formation and disassembly**

We have performed detailed stopped-flow transient kinetic investigations of the formation and disassembly of human Rad51 filaments in the absence of nucleotide and in the presence of ATP or non-hydrolyzable nucleotides or analogs (AMPPNP, ADP). Surprisingly we found that, in striking contrast to the previously characterized yeast isoform, human Rad51 filament formation does not require the presence of nucleotide.

We found that the experimental system utilizing fluorescently-labeled DNA substrates (typically, dT_{79} labeled with Cy3 at its 5' end) is suitable for monitoring these events at an advantageous signal-to-noise ratio with high temporal resolution.

b) Effect of the BLM and RecQ5β helicases and BRCA2 protein fragments on human Rad51 filament formation and disassembly

In transient kinetic experiments we showed the ability of human BLM and RecQ5β helicases and Brc repeats of the human BRCA2 protein to catalyze the disassembly of human Rad51-ssDNA nucleoprotein filaments. We also found that abolishing the interaction between RecQ5β and Rad51 via the F666A RecQ5β mutation impairs the capability of the helicase to disassemble Rad51 filaments (Fig. 1).

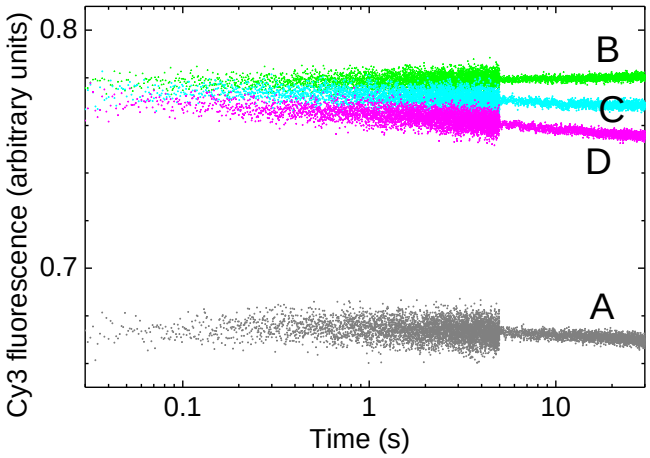

Figure 1: Stopped-flow experiments monitoring the partial disassembly of Rad51 nucleoprotein filaments via fluorescence changes of 5'-Cy3-dT_{79}. Control trace A: 40 nM 5'-Cy3-dT_{79} mixed with buffer. Control trace B: premixture of 40 nM 5'-Cy3-dT_{79}, 2 µM Rad51 and 5 mM ATP mixed with buffer. Traces C-D: 5'-Cy3-dT_{79}, Rad51 and ATP mixed with 3 µM F666A mutant (C) or wild-type (D) RecQ5β.

Aim 2: Cooperation between ssDNA binding proteins and RFHs during translocation along and unwinding of DNA strands

a) Interaction of RecQ with SSB and DNA

In these experiments we investigated the coupling between the interaction of RecQ helicase with DNA and SSB. In ammonium sulfate co-precipitation experiments, we demonstrated that RecQ interacts with SSB, and the interaction is weaker if the C-terminal peptide motif of SSB is absent (in the SSBdC8 construct). We showed that the equilibrium binding of the SSB C-terminal peptide (SSBC8) causes a decrease in RecQ's tryptophan (Trp) fluorescence intensity, which can be used as a useful signal monitoring the interaction. Stopped-flow experiments showed that the interaction is rapid and occurs on the milliseconds time scale. We also showed that the SSBC8 peptide slightly increases the DNA affinity of RecQ (Fig. 2).

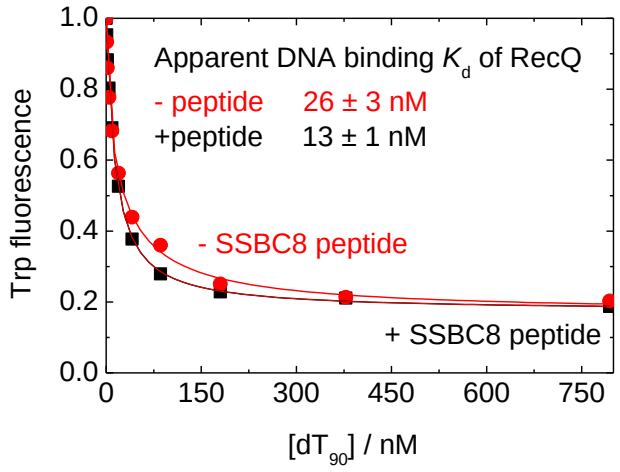

Figure 2: Trp fluorescence titration of 100 nM RecQ with dT_{90} in the absence and presence of 100 uM SSBC8 peptide

In stopped-flow transient kinetic experiments we showed that the binding of both wild-type SSB and SSBdC8 to ssDNA is rapid and diffusion-controlled. We have also characterized the DNA binding properties of a coumarin-labeled fluorescent variant of SSB (DCC-SSB), which provides a useful signal for DNA interaction experiments. We showed that DCC-SSB binds DNA with parameters similar to wild-type SSB. Synthesis of the results on the effect of the SSB interaction on RecQ's DNA binding properties is underway.

***b)* Effect of SSB on RecQ's ATPase activity**

We showed that both SSB and the SSBC8 peptide significantly inhibit the basal (DNA-free) ATPase activity of RecQ, which is a useful fingerprint signal to monitor their interaction. In the presence of DNA, we found that RecQ's ATPase activity is stimulated by low SSB concentrations, but at higher SSB concentrations this activation turns into inhibition, reflecting complex interactions between the proteins and DNA. The activation effect was not observed in the case of the SSBC8 peptide.

We observed that SSB did not completely inhibit RecQ's DNA-activated ATPase activity even at very high concentrations. On the other hand, the SSBdC8 construct exerted quasi-complete inhibition (Fig. 3). SSB and SSBdC8 showed similar DNA-binding affinities. These findings suggest that RecQ can dismantle wild-type SSB – but not SSBdC8 – from ssDNA, and the interaction between the SSB C-terminal peptide and RecQ is necessary for this activity. This conclusion was further substantiated by experiments in which the SSBC8 peptide decreased RecQ's ATPase activity to negligible levels in the presence of wild-type SSB-DNA filaments, showing that the peptide competes for the SSB-RecQ interaction (Fig. 3).

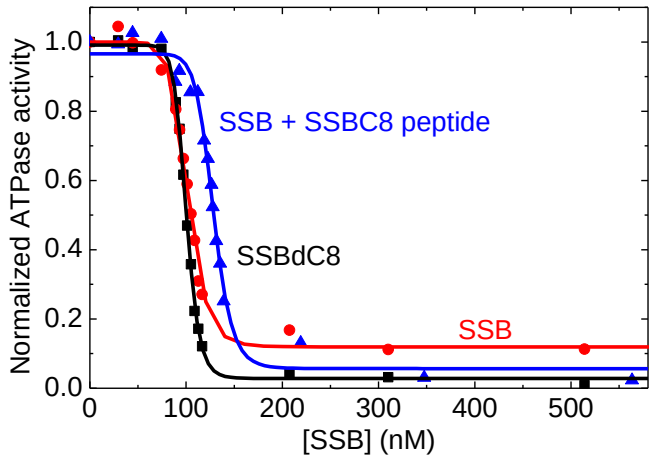

Figure 3: ATPase activity titration of 30 nM RecQ in the presence of 100 nM dT_{54}, in the presence or absence of 200 μM SSBC8 peptide and various SSB concentrations

***c)* Effect of SSB on the ssDNA translocation and DNA unwinding activities of RecQ**

In stopped-flow transient kinetic experiments monitoring RecQ's ATP hydrolytic activity via a fluorescently labeled phosphate binding protein (MDCC-PBP), we found that in the presence of SSB, RecQ translocates with a mean processive run length of 60 nucleotides per run. The data also indicate that RecQ's translocative ATPase activity is reduced when it reaches an SSB tetramer on ssDNA. During translocation, RecQ's ATPase activity is not

affected by SSB. The data showed that, in contrast to SSB, the SSBC8 peptide alone does not impose translocation terminations (Fig. 4).
In line with the above results, the ssDNA length dependence of RecQ's ATPase activity in the absence and presence of the SSBC8 peptide indicated that, in contrast to SSB, the peptide alone does not influence RecQ's ssDNA translocation activity. Analysis of the results on the effect of SSB and the SSBC8 peptide on RecQ's dsDNA unwinding activity is underway.

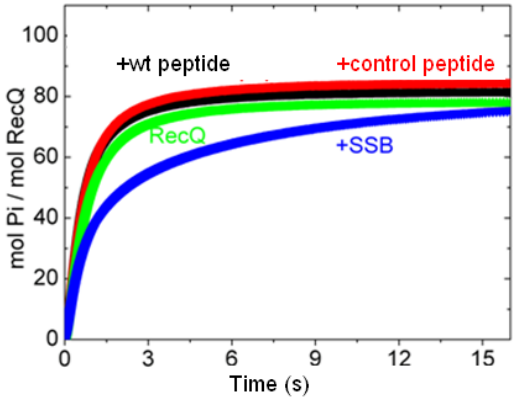

Figure 4: MDCC-PBP fluorescence stopped-flow transients monitoring ATP consumption by RecQ helicase during translocation along ssDNA. 50 nM RecQ plus 10 nM M13 circular ssDNA was rapidly mixed with 1 mM ATP and 32 mg/ml heparin (used as a protein trap to ensure single-round translocation conditions) in the absence or presence of 660 nM SSB or 120 µM SSBC8 peptide ("wt peptide") or mixed-sequence control peptide

4. PUBLICATIONS RESULTING FROM THE PROJECT

4.1 Published research article

1. Gyimesi, M., Harami, G. M., Sarlós, K., Hazai, E., Bikádi, Z., **Kovács, M.** (2012): Complex activities of the human Bloom's syndrome helicase are encoded in a core region comprising the RecA and Zn-binding domains. *Nucleic Acids Res.* 2012 Jan 16 [Epub ahead of print]

4.2 Research articles under review

1. Gyimesi, M., Pires, R.H., Billington, N., Sarlós, K., Kocsis, Z. S., Módos, K., Sellers, J. R., Kellermayer, M. S., **Kovács, M.** (2012): Monomeric form of the human Bloom's syndrome helicase is recruited to various homologous recombination intermediates
2. Sarlós, K., Gyimesi, M., **Kovács, M.** (2012): RecQ helicase translocates along single-stranded DNA with a moderate processivity and tight mechanochemical coupling

4.3 Research articles in preparation

1. Harami, G., Gyimesi, M., **Kovács, M.** (2012): Mechanism of D-loop disruption by the human Bloom's syndrome helicase
2. Harami, G., Gyimesi, M., **Kovács, M.** (2012): Roles of the winged helix domain in DNA binding proteins

3. Sarlós, K., Gyimesi, M., Kele, Z., **Kovács, M.** (2012): Insights into the DNA activation of the RecQ enzymatic cycle reveal mechanistic diversity within RecQ-family helicases
4. Harami, G., **Kovács, M.** (2012): Effect of the SSB protein on the mechanoenzymatic action of RecQ helicase
5. Spirek, M., Harami, G., Gyimesi, M., Nagy, N., Molnár, E., Krejci, L., **Kovács, M.** (2012): Effects of RecQ-family helicases on the formation and disassembly of human Rad51 nucleoprotein filaments

4.4 Conference abstracts

1. Sarlós, K., Gyimesi, M., Neuman, K.C., **Kovács, M.** (2011): Mechanochemistry of DNA helicases. *HFSP Meeting,* Montreal, Canada
2. Sarlós, K., Gyimesi, M., Neuman, K.C., **Kovács, M.** (2011): Temperature-dependent switch in the mechanochemical activity of RecQ helicases. *Central European Meeting on Genome Stability and Dynamics,* Bratislava, Slovakia
3. Gyimesi, M., Harami, G., Sarlós, K., **Kovács, M.** (2011): Basic homologous recombination activities can be performed by the minimal core of Bloom's syndrome helicase. *Central European Meeting on Genome Stability and Dynamics,* Bratislava, Slovakia
4. Kocsis, Z. S., Pintér, L., Haracska, L., **Kovács, M.** (2011): Non-canonical helicase in DNA repair. *TÁMOP Meeting,* Dobogókő, Hungary
5. Sarlós, K., Gyimesi, M., **Kovács, M.** (2011): Harnessing of chemical energy for mechanical work in DNA-restructuring enzymes. *TÁMOP Meeting,* Dobogókő, Hungary
6. Gyimesi, M., Sarlós, K., Harami, G., Kocsis, Z. S., Pires, R. H., Módos, K., Derényi, I., Kellermayer, M., **Kovács, M.** (2011): Stability and variability: genome-maintaining activities of DNA-restructuring motor enzymes. *TÁMOP Meeting,* Dobogókő, Hungary
7. Harami, G., Gyimesi, M., Sarlós, K., **Kovács, M.** (2011): Functional anatomy of the human Bloom's syndrome helicase. *FASEB Helicase Meeting,* Steamboat Springs, CO, USA
8. Sarlós, K., Gyimesi, M., Pires, R. H., Módos, K., Kellermayer, M. S. Z., **Kovács, M.** (2011): Dynamic switch between assembly states of the human Bloom's syndrome helicase during homologous recombination. *FASEB Helicase Meeting,* Steamboat Springs, CO, USA
9. Kocsis, Z. S., Pintér, L., Haracska, L., **Kovács, M.** (2011): ATPase properties of a non-canonical helicase. *FASEB Helicase Meeting,* Steamboat Springs, CO, USA
10. **Kovács, M.** (2011): Stability and variability: genome-maintaining activities of DNA-restructuring motor enzymes. *25th Anniversary of the Hungarian Scientific Research Fund (OTKA),* Budapest, Hungary
11. Gyimesi, M., Harami, G., Sarlós, K., Roy, D., **Kovács, M.** (2011): Bloom's syndrome helicase: functional anatomy of a DNA-restructuring motor protein. *Annual Meeting of the Hungarian Biochemical Society,* Pécs, Hungary
12. Kocsis, Z. S., Pintér, L., Haracska, L., **Kovács, M.** (2011): ATPase properties of a non-canonical helicase. *Annual Meeting of the Hungarian Biochemical Society,* Pécs, Hungary

13. Harami, G., Gyimesi, M., Sarlós, K., **Kovács, M.** (2011): Enzymatic processes in DNA repair: Genome maintaining activities of RecQ helicases. *4th European Conference on Chemistry for Life Sciences,* Budapest, Hungary
14. Kocsis, Z. S., Pintér, L., Haracska, L., **Kovács, M.** (2012): Mechanochemistry of the Rad5 double-stranded DNA translocase. *56th Annual Meeting of the Biophysical Society,* San Diego, CA, USA
15. Harami, G., Gyimesi, M., **Kovács, M.** (2012): Mechanism of D-loop disruption by the human Bloom's syndrome helicase. *56th Annual Meeting of the Biophysical Society,* San Diego, CA, USA
16. Gyimesi, M., Pires, R. H., Sarlós, K., Nagy, N. T., Módos, K., Kellermayer, M. S. Z., **Kovács, M.** (2012): Dynamic switch between assembly states of the human Bloom's syndrome helicase during homologous recombination. *56th Annual Meeting of the Biophysical Society,* San Diego, CA, USA
17. Gyimesi, M., Harami, G., Pires, R. H., Sarlós, K., Hegyi, G., Módos, K., Kellermayer, M. S., **Kovács, M.** (2012): Mechanism of regulation of homologous recombination by the human Bloom's syndrome helicase. *FEBS3+ Meeting,* Opatija, Croatia
18. Kocsis, Z. S., Pintér, L., Haracska, L., **Kovács, M.** (2012): Mechanochemistry of the Rad5 double-stranded DNA translocase. *FEBS3+ Meeting,* Opatija, Croatia

4.5 Media appearances

1. A motorenzimek szerepe a rákkutatásban. [Motor enzymes in cancer research.] *MTA honlap – A tudomány hírei*, July 5, 2011
2. Mozgató enzimek. [Enzymes that make things move.] *Figyelő,* 55/33. Aug 18-24, 2011
3. m1 (TV), *Delta.* Oct 22, 2011
4. Hibák a DNS-ben. [Interview: Errors in DNA.] *ELTE honlap,* February 22, 2012
5. m1 (TV), *Delta.* Apr 14, 2012