FINAL REPORT
Molecular mechanisms of genome maintenance
Project NNF78783
Project period: 01.04.2009–31.03.2011

This project was aimed at the elucidation of the mechanisms of action of DNA helicases during homologous recombination-based DNA repair processes. DNA helicases essentially contribute to the repair of DNA lesions and error-free transmission of genetic information. Their malfunctions cause high cancer predisposition and accelerated ageing. We investigated the mechanisms of action of the human BLM (Bloom syndrome) and *E. coli* RecQ helicases.

Our data provided new insights into the mechanisms of substrate recognition and oligomerisation states of BLM and RecQ (Aim 1), D-loop unwinding (Aim 2), DNA strand annealing (Aim 3), and branch migration (Aim 4).

Our experimental setup included recombinant DNA technology, transient kinetic assays using site-specific fluorescence tags, and structural studies using dynamic light scattering and atomic force microscopy.

As the BLM helicase is a key player in the maintenance of the integrity of genetic material, the obtained new insights will aid future efforts to understand and control processes that lead to a range of cancerous diseases.

PUBLISHED RESEARCH ARTICLES

1. Gyimesi, M., Sarlós, K., Derenyi, I., Kovács, M. (2010): Streamlined determination of processive run length and mechanochemical coupling of nucleic acid motor activities, Nucleic Acids Research 38:e102.

Full text: http://nar.oxfordjournals.org/content/early/2010/01/31/nar.gkq014.full

In this work we developed an analytical method suitable for the determination of all key functional parameters (enzymatic rates, processivity and mechanochemical coupling) of nucleic-acid based motor enzymes. This analysis is suitable for rapid and precise assessment of the effects of mutations, physical conditions, binding partners and other effectors on the functioning of translocases, helicases, polymerases and other NTP-consuming processive nucleic acid motors.

This work is related to all Aims of the project.

2. Gyimesi, M., Sarlós, K., Kovács, M. (2010): Processive translocation mechanism of the human Bloom's syndrome helicase along single-stranded DNA, Nucleic Acids Research 38:4404-14.

Full text: http://nar.oxfordjournals.org/content/early/2010/03/08/nar.gkq145.full

In this work we presented a quantitative model for the translocation of a monomeric form of the BLM helicase (BLM^{1290}) along ssDNA. We showed that BLM performs translocation at a low mechanochemical coupling ratio (1 ATP consumed/1 nucleotide traveled) and moderate processivity (with a mean number of 50 nucleotides traveled in a single run). We also showed that the rate-limiting step of the translocation cycle is a transition between two ADP-bound enzyme states, and concluded that BLM performs double-stranded DNA unwinding by fully active duplex destabilization.

This work is related to Aims 1, 3, and 4 of the project.

RESEARCH MANUSCRIPTS AWAITING SUBMISSION

3. Sarlós, K., Gyimesi, M., Kele, Z., Kovács, M.: Mechanoenzymatic mechanism of Escherichia coli RecQ helicase

Maintenance of genome integrity, which is essential for survival of cells, is the major biological role of *E. coli* RecQ helicase via its participation in homologous recombination (HR) mediated DNA repair processes. RecQ exerts its functions by utilizing the free energy of ATP hydrolysis for mechanical movement along DNA tracks (translocation), which also serves as a basis for strand separation (unwinding). The coupling between the ATPase activity and the DNA interaction of the enzyme is essential in order to complete several successive translocation steps without detachment from the DNA track. In this work we present a unified mechanistic model of the coupling of the ATPase cycle to translocation via periodically modulating the strength of the enzyme-DNA interaction. We show that the rate-limiting step of the ATPase cycle is the chemical hydrolysis step, which is kinetically activated by DNA around 300 times. We detected two (a weak and a strong) DNA-bound conformations of RecQ. We found that an enzymatic state whose formation is coupled to ATP hydrolysis adopts a DNA-clamped conformation with an extremely high DNA affinity. This finding implies that the chemical hydrolysis step may be linked to a mechanical step along DNA. The predominance of the strongly DNA-bound conformation results a relatively high processivity (around 330 steps/run), while the enzyme translocates with 1-nucleotide step size at a low mechanochemical coupling ratio (1 ATP consumed/nucleotide traveled).

This work is related to Aims 1, 3, and 4 of the project. Two key figures of the manuscript are shown below.

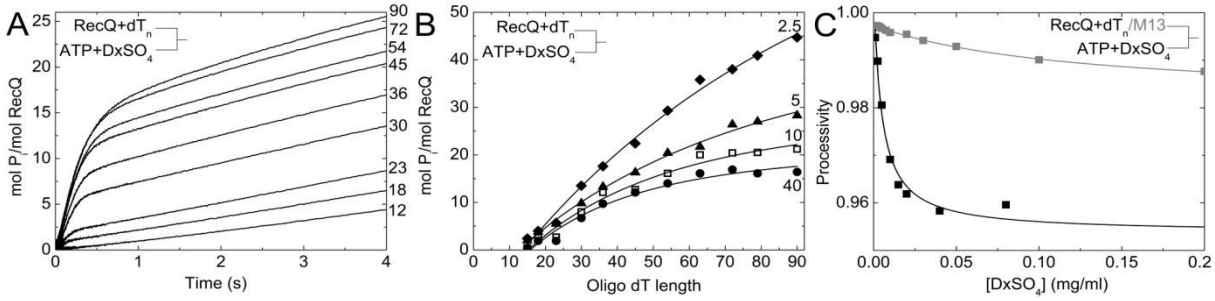

Figure 1: Inorganic phosphate (P_i) production from ATP hydrolysis during single-round translocation of RecQ helicase along single-stranded (ss) DNA

A, Time courses of P_i production during translocation of RecQ along ssDNA substrates of different length (dT_{12} – dT_{90}, lengths in nt indicated), recorded upon mixing 25 nM RecQ plus 1 μM dT_n with 0.5 mM ATP plus 0.04 mg/ml dextran sulphate ($DxSO_4$, used as a protein trap to provide single-round translocation conditions) in the stopped-flow instrument. P_i production was monitored by utilizing the fluorescence change of a fluorescently labeled P_i binding protein (MDCC-PBP, 5 μM in all syringes). ***B,*** Oligo-dT length dependence of the amplitude of P_i production (mol P_i/mol RecQ) during the first phase, corresponding to translocation along ssDNA. Indicated values are $DxSO_4$ concentrations in μg/ml. ***C,*** Dependence of the determined processivity on $DxSO_4$ concentration during translocation along linear oligo-dT (black) and circular M13 phage ssDNA (grey). Equations used in the fits were described in Paper 1 (see Published Research Articles).

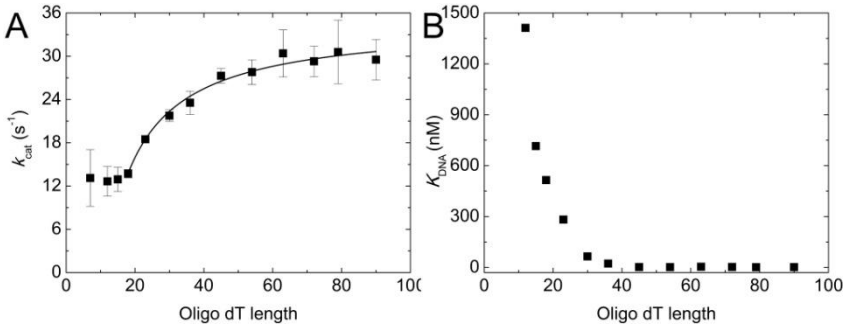

Figure 2: Oligo-dT length dependence of the DNA-activated steady-state ATPase activity of RecQ helicase
A, k_{cat} values shown were determined from oligo-dT concentration dependence of the steady-state ATPase activity of RecQ. The equation used in the fit was described in Paper 2 (see Published Research Articles). ***B,*** Dependence of K_{DNA} values (oligo-dT concentrations required for half-saturation) on oligo-dT length.

4. Gyimesi, M., Pires, R. H. J., Sarlós, K., Módos, K., Kellermayer, M. S. Z., Kovács, M.: Substrate-induced dynamic oligomerisation of the human Bloom's syndrome helicase during homologous recombination

Homologous recombination (HR) is the key process in the repair of double-stranded DNA breaks (DSB), the most severe form of DNA damage that can lead to cancer or cell death. The human Bloom's syndrome helicase (BLM) plays essential pro- and anti-recombinogenic activities, which must be balanced to maintain efficient DSB repair while avoiding illegitimate recombination events. Previously it was thought that the full-length BLM helicase generally functions in an oligomeric form. By solution and single-molecule analyses, we made the striking discovery that the active form of full-length BLM during ATP hydrolysis is monomeric both in the absence of DNA and in the presence of single-stranded DNA substrates. However, complex DNA structures resembling recombination intermediates induce multimerization of BLM. These results led to a model in which BLM dynamically switches between assembly states depending on the DNA structure encountered during various stages of HR.

This work is related to Aims 1, 2, and 4 of the project. Two key figures of the manuscript are shown below.

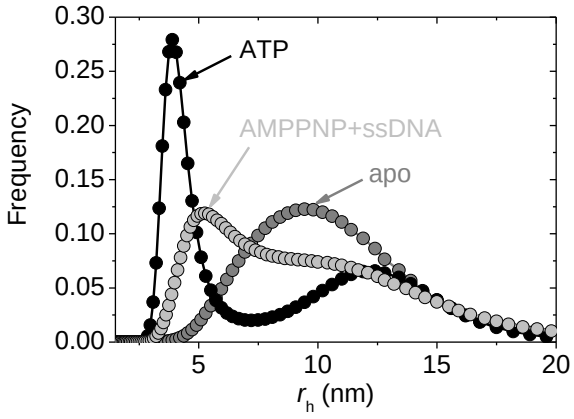

Figure 3: Dynamic light scattering (DLS) spectra of full-length and truncated ($BLM^{642-1290}$) BLM helicase constructs recorded in different conditions
DLS spectra of full-length BLM in apo (dark grey), ATP (black), and AMPPNP plus dT_{54}-bound (light grey) states are shown. Exponentially modified Gaussian functions were fitted to the data points.

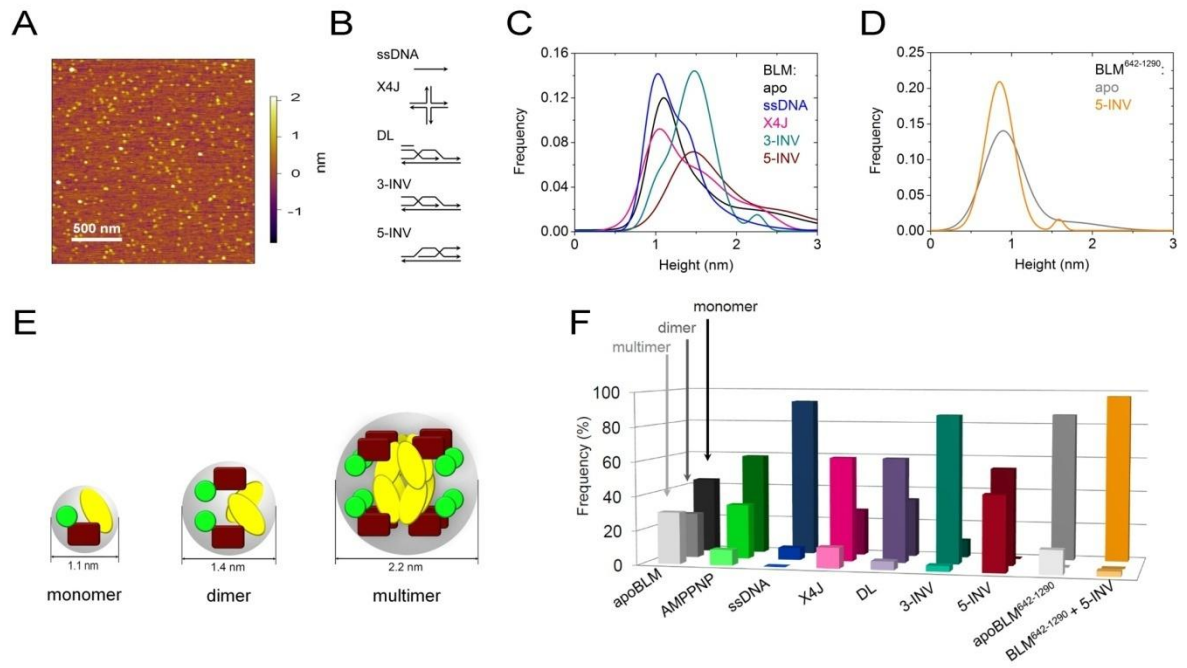

Figure 4: Scanning-mode atomic force microscopic (AFM) analysis of single BLM helicase particles
A, Representative 2x2-µm portion of a 5x5-µm AFM scan of BLM in the presence of AMPPNP and D-loop (DL) substrate. ***B,*** Schematic illustration of DNA substrates used in this study. Arrows represent one strand of DNA pointing toward the 3'-end. ***C-D,*** Gaussian fits to histograms resulting from height analysis of BLM and $BLM^{642\text{-}1290}$ AFM images recorded in the presence of various substrates. Three major populations were distinguished in BLM with heights of 1.1 ± 0.1 nm, 1.4 ± 0.03 nm, and 2.2 ± 0.1 nm. ***E,*** Schematic model of BLM oligomerization states used to interpret AFM particle height data. Full-length BLM consists of a large N-terminal domain (yellow), the conserved helicase core consisting of RecA-like folds (brown), and the C-terminal region comprising the RecQ C-terminal plus helicase and RNase D C-terminal (HRDC) domains (green). We identified the 1.1-nm population as the monomeric form because monomeric $BLM^{642\text{-}1290}$ and γ-globulin exhibited similar height values. The 1.4-nm population is proposed to be dimeric ($V_{1.4nm}$ = 2.1x $V_{1.1nm}$), whereas the widely distributed 2.2-nm population is probably a mixture of higher-order oligomers ($V_{2.2nm}$ = 8.0x $V_{1.1nm}$). ***F,*** Column representation of the distribution of BLM and $BLM^{642\text{-}1290}$ between monomeric, dimeric and higher-order oligomeric states.

5. Gyimesi, M., Harami, G., Sarlós, K., Kovács, M.: A minimal functional unit of the human BLM helicase

In this study we aimed at the precise identification of the roles of different domains of the BLM helicase in its enzymatic activities exerted during DNA repair processes. These activities include translocation on single-stranded DNA, annealing and unwinding of the strands of double-stranded DNA, and the disruption of Rad51 nucleoprotein filaments and D-loops. By truncation studies, we also searched for the minimal functional unit of the helicase. Two hairpin-like motifs of RecQ-family helicases had previously been implicated as critical for DNA unwinding; one is located in one of the two tandem RecA-like domains (RecA pin), whereas the other is in the family-specific RecQ-C-terminal domain (RQC pin). However it was not known which of these pins is critical for activity in BLM.

Our results surprisingly showed that a BLM construct consisting solely of the two RecA domains and the Zn^{2+}-binding domain (BLM^{1077}) retained all basic mechanochemical activities of the full-length enzyme. In contrast to the longer, previously-characterized BLM^{1290} construct, BLM^{1077} restored the Rad51 nucleoprotein filament clearing activity of full-length BLM. The results also demonstrated that the RecA pin is sufficient for strand separation by the BLM helicase.

This work is related to Aims 2, 3, and 4 of the project. Three key figures of the manuscript are shown below.

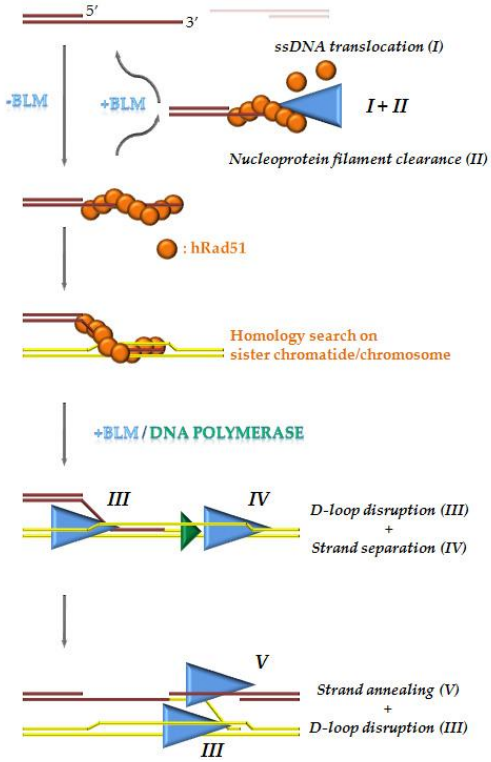

Figure 5: Various mechanochemical activities of BLM helicase (blue) during homologous recombination

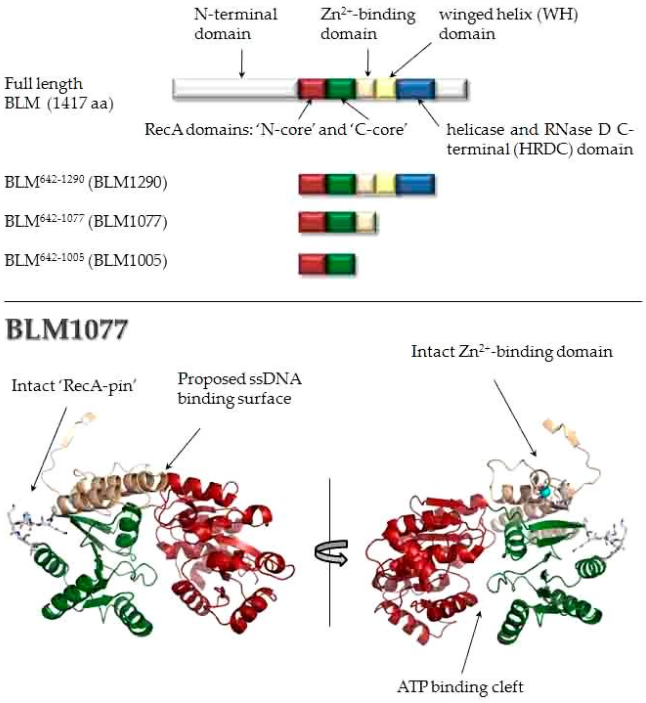

Figure 6: Domain structure of full-length and truncated BLM helicase constructs used in this study

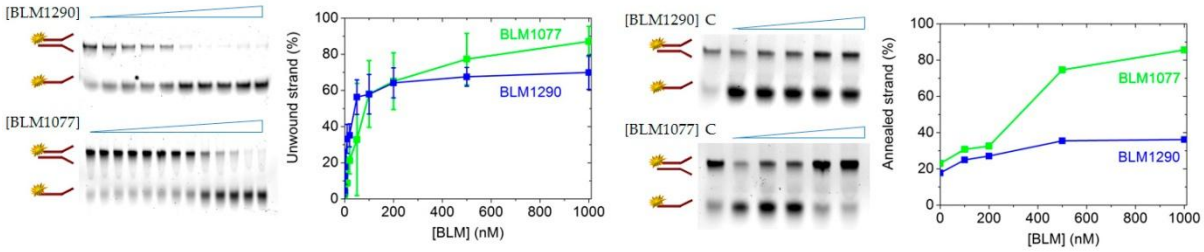

Figure 7: BLM^{1077} unwinds and anneals splayed-arm DNA substrate more effectively than BLM^{1290}
Gel images and corresponding graphs show results of double-stranded DNA unwinding (left) and strand annealing (right) experiments. The fluorescent signal of fluorescein-labeled DNA was used in these experiments. 3 nM splayed arm DNA (unwinding) or 3-3 nM ssDNA (forming splayed arm; annealing) substrates were titrated with BLM at different concentrations (15 min, 37°C) and run on a 12 % non-denaturing gel. (C: control splayed arm DNA)

CONFERENCE PROCEEDINGS

1. Gyimesi, M., Sarlós, K., Kovács, M.: Processive translocation mechanism of the human Bloom's syndrome helicase along single-stranded DNA, *EMBO Meeting on Helicases and Nucleic Acid Machines,* Les Diablerets, Switzerland, 2009

2. Sarlós, K., Gyimesi, M., Kovács, M.: Mechanism of DNA-dependent enzymatic activation of *Escherichia coli* RecQ helicase, *EMBO Meeting on Helicases and Nucleic Acid Machines,* Les Diablerets, Switzerland, 2009

3. Gyimesi, M., Sarlós, K., Kovács, M.: A humán Bloom szindróma helikáz processzív transzlokációs mechanizmusa, *A Magyar Biokémiai Egyesület Vándorgyűlése,* Budapest, 2009 [Processive translocation mechanism of the human Bloom's syndrome helicase, *Annual Meeting of the Hungarian Biochemical Society,* Budapest, 2009]

4. Sarlós, K., Gyimesi, M., Kovács, M.: A DNS-függő enzimaktiváció szerepe a RecQ helikáz működésében, A Magyar Biokémiai Egyesület Vándorgyűlése, Budapest, 2009 [Role of DNA-dependent enzymatic activation in the functioning of RecQ helicase, *Annual Meeting of the Hungarian Biochemical Society,* Budapest, 2009]

5. Gyimesi, M. Sarlós, K., Harami, G., Kocsis, Z., Kovács, M.: Deciphering the mechanochemistry of RecQ-family DNA helicases, *EMBO DNA Repair Meeting,* Brno, Czech Republic, 2010

6. Sarlós, K., Gyimesi, M., Kovács, M.: Mechanism of DNA-dependent enzymatic activation of *Escherichia coli* RecQ helicase, *54th Annual Meeting of the Biophysical Society,* San Francisco, CA, USA, 2010

7. Gyimesi, M., Sarlós, K., Kovács, M.: Two RecA domains comprise a minimal functional unit of the human BLM helicase, *55th Annual Meeting of the Biophysical Society,* Baltimore, MD, USA, 2011

PUBLIC SCIENCE WRITING (IN HUNGARIAN)

1. Sarlós Kata, Gyimesi Máté, Kovács Mihály: Anyagmozgatás és információ-továbbítás a sejtben: biológiai motorok. Természet Világa 2009. május [Material transport and information processing in the cell: biological motors]
Full text: http://www.mk-lab.org/downloads/sarlos_termvil_2009_majus.pdf

2. Gyimesi Máté, Vellai Tibor, Kovács Mihály: A genetikai állomány stabilitása: helikáz enzimek szerepe a DNS-hibajavításban, Természet Világa, 2010. március, 2010 [Stability of the genetic material: role of helicase enzymes in DNA repair]
Full text: http://www.mk-lab.org/downloads/gyimesi_termvil_2010.pdf

PRIZES THAT RESULTED FROM THE PROJECT

1. November 2009: Gábor Harami was awarded 1st Prize at the Student Research Conference of Eötvös University for his work entitled "Interaction between the *E. coli* RecQ helicase and SSB protein during genome maintenance"

2. May 2010: Gábor Harami received the Student Excellence Award of Eötvös University for his work entitled "Interaction between the *E. coli* RecQ helicase and SSB protein during genome maintenance"

3. June 2010: Mihály Kovács received the Bolyai Fellowship of the Hungarian Academy of Sciences.

4. August 2010: Dr. Máté Gyimesi (participant of the project) received the Prize of the Hungarian Biochemical Society for his work performed in this project

PUBLIC APPEARANCES RELATED TO THE PROJECT

1. February 2010: Kovács Mihály: Hibajavító fehérjemotorok, OTKA honlap - A hónap kutatója [Motor proteins in DNA repair. *Researcher of the Month, Hungarian Scientific Research Fund*]
Full text: http://www.otka.hu/index.php?akt_menu=4121

2. November 2010: Máté Gyimesi's lecture entitled "Working mechanisms of molecular motors" at Eötvös Collegium, Budapest

3. November 2010: Mihály Kovács' lecture entitled "Helicases: Motors of DNA rearrangement" at Symposium on Protein Flexibility of the Hungarian Academy of Sciences