Mechanobiochemistry of RecQ helicases

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Theses

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INTRODUCTION

The information of life is encoded in the DNA molecule which has a stable, closed structure established for the long-term storage of information. During normal, physiological functioning of living organisms, DNA is exposed to several damaging agents such as reactive oxygen species produced by cell metabolism, UV irradiation, etc.), which can lead to different types of DNA damages. These damages are repaired by evolutionarily conserved, specific repair pathways based on the concerted action of dedicated enzyme complexes. One of the most toxic forms of DNA damages is the double-stranded break, which can induce cell death even in small numbers.

Homologous recombination (HR) is an evolutionarily conserved process, which plays a dual role in the living organisms. During meiosis HR leads to the shuffling of the maternal and paternal information. On the other hand, HR allows the error-free, information conserving repair of DNA double-stranded breaks. The unwinding of the strands of double-stranded (ds) DNA is essential during several steps of HR. The enzymes, which are capable to separate the strands of the dsDNA, are called helicases. Helicases are ubiquitous motor enzymes, utilizing the chemical energy stored in nucleotide triphosphates (mostly ATP) to travel unidirectionally along single-stranded (ss) DNA (RNA in some cases), which leads to the separation of the two strands of the dsDNA from each other.

RecQ helicases play key roles in HR. They are widespread from the bacterial *Escherichia coli* RecQ to the five human forms. Their importance is reflected in the fact that the loss-of-function mutations of three of the five human forms lead to severe autosomal hereditary diseases, associated with high cancer predisposition. These deficiencies are the Bloom's syndrome (BLM helicase), the Werner's syndrome (WRN helicase), and the Rothmund-Thomson syndrome (RecQL4).

E. coli RecQ and human BLM are important players in the early, quality control phase of HR. During this process they unwind non-allelic or illegitimate recombination intermediates. On the other hand RecQ helicases promote the processing of HR intermediates during later HR processes. The (double) Holliday junction, which is a central intermediate of HR, is resolved by RecQ helicases resulting only non-crossover products. Besides double-stranded break repair, RecQ and BLM are key players in the stabilization and HR-mediated processing of the stalled replication forks.

RecQ helicases comprise two RecA folds forming the nucleotide-binding cleft. This structural element is the general basis of mostly all helicases (AAA^+ domains replace the

RecA domains in some cases), which is the proof of their common origin. The so-called RecQ-C-terminal domain is characteristic to the RecQ family. This domain comprises a structure-stabilizing zinc-binding (ZB) domain, and a winged-helix domain, proposed to be responsible for protein-protein or protein-DNA interactions. RecQ helicases also possess a so-called HRDC (Helicase/RNase D C-terminal) domain, which role is not yet cleared. *E. coli* RecQ helicase, and the helicase module of the BLM helicase (BLM^{HM}, containing the amino acids 642-1290) is built up from the above mentioned domains. The full-length BLM comprises more structural elements which are responsible for protein-protein interactions, oligomerisation, or nuclear localisation signal. The apo and ATP-γ-S-bound crystal structure of *E. coli* RecQ lacking the HRDC domain has been solved, but there is no crystal structure of the helicase module of BLM. Furthermore there is no crystal structure of the DNA-bound form of neither RecQ nor BLM. Therefore we can only deduce the exact DNA-binding site and the structural elements responsible for unwinding or translocation from functional tests.

The *in vivo* and biochemical properties of RecQ and BLM helicases has been widely investigated. On the other hand a comprehensive model about the coupling of the fundamental activities (ATPase, translocase, helicase) is still lacking. Our working hypothesis is based upon the idea, that the quantitative understanding of the molecular mechanism of RecQ helicases will answer questions which was unattainable using other approaches.

AIMS

Our aim was to understand the molecular mechanism of RecQ and BLM. In detail, we wanted to reveal the mechanism of the coupling of ATPase activity to interaction with DNA, resulting processive translocation. We assessed this issue with the following approaches:

- We dissected the ATPase cycle of RecQ and BLM to individual steps, and investigated the effect of DNA on their kinetics.
- We investigated the kinetics of the RecQ-DNA interaction in different nucleotide states.
- We developed a streamlined method to characterize the translocation of any NTP-driven nucleic-acid motor protein.
- Based on the above mentioned and complementary methods we characterized the translocation of RecQ and BLM along ssDNA.

- We validated the comprehensive mechanochemical model of the working of RecQ helicase based on Global kinetic modelling using the experimentally determined parameters.

APPLIED TECHNIQUES

- Cloning of recombinant protein constructs. PCR amplification of RecQ and BLM^{HM} from genomic DNA. Insertion of constructs into pTXB3 plasmid between NcoI and LguI restriction sites.
- Expression of RecQ and BLM^{HM} proteins in *E. coli* B ER2566 and *E. coli* BL21 Rosetta cells, respectively.
- Protein purification:
    - Affinity chromatography – Intein-chitin-binding-domain – chitin column (RecQ, BLM^{HM})
    - Affinity chromatography – heparin column (RecQ, BLM^{HM})
    - Ion exchange chromatography – CM column (BLM^{HM})
    - Ion exchange chromatography – Q Sepharose FF (Phosphate binding protein - PBP)
- Modification of proteins: labelling of PBP with MDCC (7-Diethylamino-3-((((2-Maleimidyl)ethyl)amino)carbonyl)coumarin)
- Steady-state kinetic measurements using PK/LDH coupled assay (basal and DNA-activated; Shimadzu UV-2101PC spectrophotometer)
- Fluorescence Equilibrium Titration – RecQ tryptophan (Trp) fluorescence (SPEX Fluoromax spectrofluorometer)
- Rapid kinetic measurements
    - KinTek-2004, BioLogic SFM-300/400
    - Fluorescence signals:
        - Trp – excitation: 280 nm and 297 nm, filter: 320 LP, 340 IF
        - MDCC-PBP – excitation: 436 nm, filter: 455 LP
        - mdATP/ADP ((3'-(N-methylanthraniloyl)-2'-deoxy-ATP and -ADP) – excitation: 280 nm, filter 420 LP
- Oxygen exchange experiments
- SDS-PAGE

RESULTS (THESES)

- We have developed a streamlined method for the characterization of the unidirectional movement of any NTP-driven nucleic acid motor enzyme.
- Based on the above mentioned and other complementary techniques, we characterised the translocation of BLM^{HM} helicase along ssDNA:
    - BLM^{HM} occludes 12-14 nucleotides upon randomly binding to ssDNA.
    - BLM^{HM} translocates along ssDNA with an inchworm type mechanism by consuming 1 ATP molecules upon one nucleotide travelled.
    - The enzyme performs a mean of 50 ATPase cycle during a single run.
    - We determined the key rate constants of the translocation of BLM^{HM} (translocation rate, ATPase activity during translocation and on the 5' end, rate constant of dissociation from the internal sites and from the end of ssDNA).
- We dissected the ATPase cycle of RecQ to individual steps:
    - Fast and reversible nucleotide (ATP, ADP) binding is unaffected by DNA.
    - In the absence of DNA the rate-limiting step of the ATPase cycle is the irreversible chemical hydrolysis step, which is accelerated more than 100 times by ssDNA, still remaining rate limiting.
    - Phosphate release is fast and irreversible.
- We characterised the RecQ-DNA interaction:
    - The two-step DNA binding comprises a fast association and a slow isomerisation step.
    - The allosteric effect of the bound nucleotide has a significant influence on the kinetics of DNA binding and release.
    - The possibly post-hydrolytic $ADP.AlF_4$ induces the formation of a closed structure, in which DNA binding and release is significantly hindered. (It is presumably a "DNA-clamped" state.) This indicates that the hydrolysis step is coupled to a structural rearrangement within the enzyme molecule resulting in the strengthening of the DNA affinity. This process is presumably coupled to a mechanical step along ssDNA.
- Based on the above mentioned and other complementary techniques, we characterised the translocation of RecQ helicase along ssDNA:
    - RecQ occludes 18 nucleotides.

- RecQ travels one nucleotide upon consuming one ATP molecule suggesting an inchworm type of mechanism
- The enzyme performs a mean of 110-350 ATPase cycle during a single run.
- We determined the key rate constants of the translocation of RecQ (translocation rate, ATPase activity during translocation and on the 5' end, rate constant of dissociation from the internal sites and from the end of ssDNA).

- We constructed a comprehensive mechanochemical model about the working mechanism of RecQ helicase, based on the experimentally determined parameters of the ATPase cycle, the DNA-interaction, and the translocation. We validated the model by Global kinetic simulations

CONCLUSIONS

- We developed a streamlined method for the mechanochemical characterization of nucleic acid motors.
- Using this approach we characterized the translocation of RecQ and BLM along ssDNA, which revealed differences in the mechanism. These differences reflect the functional adaptation to exert specialized activities rather than evolutionary relations.
- The rate-limiting step of the ATPase cycle of RecQ is the chemical hydrolysis step in the absence and presence of DNA, in spite of the significant activation of this step by ssDNA.
- The hydrolysis step is coupled to a conformational change, which leads to a closed, "DNA-clamped" state in the post-hydrolytic ($ADP.P_i$) state. This structural rearrangement is supposedly coupled to a mechanical, translocation step along ssDNA.

PUBLICATIONS CONCERNING THE THESIS

I. Scientific publications

Sarlós, K, Gyimesi, M, Kovács, M (2012) RecQ helicase translocates along single-stranded DNA with a moderate processivity and tight mechanochemical coupling. *Proc Natl Acad Sci U S A* (Accepted manuscript).

Gyimesi, M, **Sarlós, K**, Kovács, M (2010) Processive translocation mechanism of the human Bloom's syndrome helicase along single-stranded DNA. *Nucleic Acids Res* 38:4404-4414.

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

II. Conference proceedings

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

Gyimesi, M., **Sarlós, K.,** Kovács, M. (2009): Mechanism of translocation of the BLM helicase along DNA. *Central-Eastern European INSTRUCT Meeting, Budapest*

Gyimesi, M., **Sarlós, K.,** Kovács, M. (2009): Mechanism of translocation of the BLM helicase along DNA. *EMBO Young Investigator Meeting, Istanbul, Turkey*

Gyimesi, M., **Sarlós, K.,** Kovács, M. (2009): 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*

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

Gyimesi, M., **Sarlós, K.,** Kovács, M. (2009): Processive translocation mechanism of the human Bloom's syndrome helicase.*Annual Meeting of the Hungarian Biochemical Society, Budapest, Hungary*

Sarlós, K., Gyimesi, M., Kovács, M. (2009): Mechanism of DNA-dependent enzymatic activation of Escherichia coli RecQ helicase. *Annual Meeting of the Hungarian Biochemical Society, Budapest, Hungary*

Gyimesi, M., **Sarlós, K.,** Kovács, **M.** (2009): Working mechanism of the human Bloom's syndrome helicase. *53rd Annual Meeting of the Biophysical Society, Boston, MA, USA*

(The presenting author is underlined)