The role of yeast Mgs1 and human WRNIP1 proteins in replication of G-quadruplex-forming DNA sequences

Ph.D. thesis summary

Ágnes Tóth

Supervisors:
Péter Burkovics Ph.D.
senior research associate

Lajos Haracska Ph.D., D.Sc.
scientific adviser

Biological Research Centre
Institute of Genetics

Ph.D. School in Biology
University of Szeged
Faculty of Science and Informatics

Szeged
2020

Introduction

The precise and error-free replication of the genetic material is important for cells, because stalled replication fork can lead to formation of mutations, strand breaks and genomic re-arrangements, and as a result it can lead to cancerous cell formation and cell death. Replication can be hindered by several factors. These can be DNA errors from exogenous and endogenous sources, DNA strand breaks and crosslinks, as well as stable secondary DNA structures which differ from double helix. G-quadruplex (G4) is one of the best known stable secondary DNA structure, which can be formed on single-stranded guanine-rich DNA. G4s have several known biological functions, such as protection of telomeres, IgG immunoglobulin recombination, they can be found near replication origins in human, and G4s can regulate transcription and translation level of certain genes. Genomic distribution of G4 sequences is not random, and these sequences show higher conservation throughout evolution than the neighbouring locations. In the human genome G4 sequences can be found in oncogene promoters. If a mutation occurs in these sequences, cellular level of these genes can increase. This is one of the reasons why the precise replication of these sequences is extremely important. However, as G4s can form stable secondary structures they can hinder the replication fork. There are helicases and polymerases which are known to participate in unwinding and replication of G4 sequences, but the exact mechanism and the regulatory players of the process are not known yet.

The *S. cerevisiae* Mgs1 (Maintenance of genome stability 1) protein and its *H. sapiens* homologue, the WRNIP1 (Werner interacting protein 1) protein were promising candidates, as potential players in G4 replication based on several factors. The Mgs1 protein has important role in maintenance of genome stability in yeast. Both proteins are tightly connected to replication apparatus proteins: among others, polymerase δ and PCNA as well. Furthermore, the WRNIP1 protein also interacts with the WRN helicase and with TLS polymerases. Both proteins are known to interact with different types of DNA structures, however their G4-binding activity has not been studied yet. Additionally, our collaborators from Germany showed in a yeast one-hybrid experiment that the Mgs1 protein interacts with G4-contacting regions *in vivo*. Based on these facts our research focused on characterization of Mgs1 and WRNIP1 proteins in G4 replication.

Aims

Our aim was to understand the replication of G4 DNA sequences. We were looking for new players in this process. We identified the *S. cerevisiae* Mgs1 protein and its *H. sapiens* homologue, the WRNIP1 protein as protentional players, and studied their role in G4 replication. In order to answer our questions, the following experiments were performed:

S. cerevisiae Mgs1 protein:

- Characterization of DNA-binding ability of Mgs1 protein with G4 DNA substrates *in vitro* (EMSA and fluorescence anisotropy experiments).
- Characterization of ATPase activity of Mgs1 protein in the presence of G4 DNA substrate *in vitro*.

H. sapiens WRNIP1 protein:

- Characterization of DNA-binding ability of WRNIP1 protein with G4 DNA substrates *in vitro* (EMSA and fluorescence anisotropy experiments).
- Characterization of G4-unwinding activity of WRNIP1 protein *in vitro* in FRET experiment.
- Characterization of the role of WRNIP1 protein in maintenance of genome stability *in vivo*.
- Characterization of the role of WRNIP1 protein in regulation of replication speed *in vivo*.

Methods

- In order to create the plasmid constructions necessary for purification of yeast Mgs1 and human WRNIP1 proteins restriction digestion, LR reaction, ligation, plasmid purification, bacterial and yeast transformation was used.
- The proteins were purified with GST affinity chromatography, the purification was verified with gel electrophoresis using denaturing polyacrylamide gel.
- DNA binding affinity of GST-Mgs1 and GST-WRNIP1 proteins *in vitro* was verified with EMSA (electrophoretic mobility shift assay) method using native polyacrylamide gel.
- The precise DNA-binding constant (K_d) of GST-Mgs1 and GST-WRNIP1 proteins *in vitro* was determined via fluorescence anisotropy method.
- The ATPase activity of GST-Mgs1 protein *in vitro* was determined via pyruvate-kinase lactate-dehydrogenase experiment.
- The G4-altering activity of GST-WRNIP1 *in vitro* was characterized via FRET method.
- The effect of WRNIP1 on genome stability *in vivo* was characterized via quantification of γH2AX and chromosome aberration.
- The role of WRNIP1 in G4 replication *in vivo* was characterized via following of EdU/IdU incorporation.

Results

S. cerevisiae Mgs1 protein:

The Mgs1 protein preferentially binds G4 DNA substrates compared to their control pairs *in vitro*

The interaction between the protein and fluorescently labelled MYC G4 substrate, as well as control substrates, was investigated in EMSA experiment using purified protein. The purified protein preferentially binds G4-containing substrates compared to their control pairs in single-stranded and partial duplex form as well.

Afterwards, fluorescence anisotropy was used to determine the precise dissociation constant (K_d), which confirmed the EMSA results. MYC G4 and different control sequences were used as substrates in the experiment. The dissociation constant of Mgs1 towards single-stranded G4 substrate was 7 ± 2 nM, and towards control substrates it was 33–40-times weaker. The K_d value towards partial duplex G4 substrate was 24 ± 4 nM, towards control substrates it was 3–8-times weaker.

The G4 motif does not affect the ATPase activity of Mgs1 protein *in vitro*

The ATPase activity of Mgs1 was examined in pyruvate-kinase lactate-dehydrogenase experiment in the presence of MYC G4 and control substrates. The G4 motif does not significantly affect the ATPase activity compared to the control substrate.

H. sapiens WRNIP1 protein:

WRNIP1 protein preferentially binds G4 DNA substrates *in vitro*

DNA binding affinity of purified GST-WRNIP1 protein was first examined in EMSA experiment with MYC and CEB G4 substrates. The proteins preferentially bind both G4 substrate type compared to their control pairs. Afterwards, the precise dissociation constant (K_d) was determined via fluorescent anisotropy measurements. The protein preferentially binds both types of G4 substrates compared to their control pairs in single-stranded and partial duplex form as well.

The WRNIP1 protein changes the structure of G4 *in vitro*

We examined in FRET experiment if the WRNIP1 protein is able to change the structure of CEB G4. As a control a known G4-unwinding helicase was used, the BLM helicase. Our results showed that the WRNIP1 protein slightly changes the G4 structure, however it is substantially lower activity than the helicase activity of BLM.

The WRNIP1 protein has a role in the regulation of replication speed *in vivo*

Control and WRNIP1-depleted cells were treated with PhenDC3, a widely used G4-stabilizing molecule, and incorporation of EdU and IdU was followed. Results of the experiments showed that in WRNIP1-depleted cells replication speed greatly decreased upon PhenDC3-treatment compared to control cells.

The WRNIP1 protein has an important role in the maintenance of genome stability *in vivo*

The amount of double-strand breaks was followed by measuring the γH2AX intensity in WRNIP1-silenced cells upon PhenDC3-treatment. Our result showed that the amount of γH2AX significantly increased compared to the control cells.

Afterwards, the chromosome structure was examined in order to verify how the high amount of double-stranded breaks affect the genome integrity. Control and WRNIP1-silenced cells were treated with PhenDC3, chromosomes were prepared and the number of aberrant chromosomes was counted. Our results showed that in absence of WRNIP1 the number of aberrant chromosomes was increased upon PhenDC3-treatement.

In summary, we identified the yeast Mgs1 protein and its human homologue, the WRNIP1 protein, as new players in G replication. It was shown in EMSA and fluorescence anisotropy experiments that both proteins preferentially bind G4 structures *in vitro*. Furthermore, the WRNIP1 protein modified the CEB G4 structure *in vitro* in FRET experiment. Additionally, the WRNIP1 protein has an important role *in vivo* in the maintenance of genome stability and in smooth replication, as upon PhenDC3-treatement in the absence of WRNIP1 the number of double-strand breaks increased, and as a result the amount of chromosome aberrations also increased, and the replication speed decreased.

Acknowledgment

I would like to thank my supervisors Péter Burkovics and Dr. Lajos Haracska for the opportunity to conduct my experiments, for their support and indispensable help.

I would like to thank Dr. Gábor Harami and Dr. Mihály Kovács, as well as the Motor Enzymology group for their help in fluorescence anisotropy, ATPase and FRET experiments. I am grateful to Dr. Szilvia Juhász and Dr. Gyula Timinszky for their help in *in vivo* experiments, for Dr. Ilona Pfeiffer for her help in protein purification, and for Dr. Zoltán Kóta for his help in FRET measurements.

I am grateful for Dr. Tibor Pankotai and Dr. Dávid Szüts for carefully reading my thesis in a short time.

I would like to thank Dr. Enikő Sajben-Nagy and Karola Almási for their help and support while writing my thesis. I am also thankful to Katalin Kovács for her technical help, and for the help of Mutagenesis and Carcinogenesis research group.

I am grateful to Dr. Miklós Erdélyi and to the members of Institute of Genetics of Biological Research Centre to providing the necessary background for conducting my research.

I would like to thank my previous teachers for showing me the beautiful world of life sciences, especially to Ildikó Máriás.

I am grateful to Orsolya Frittmann, Eszter Sági-Zsigmond and Ildikó Kovács for their help and support during hard times. Last, but not least, I am thankful for my family, partner and friends for their support throughout my Ph.D. studies.

List of publications

MTMT identifier: 10055161
Total impact factor: 9.852

List of publications:

Theresa Zacheja*, **Ágnes Tóth***, Gábor M. Harami, Qianlu Yang, Eike Schwindt, Mihály Kovács, Katrin Paeschke, Péter Burkovics: Mgs1 protein supports genome stability via recognition of G-quadruplex DNA structures, FASEB J. 34 (9): 12646-12662 (2020). MTMT: 31397391; IF: 5.391

Ágnes Tóth*, Lili Hegedűs*, Szilvia Juhász, Lajos Haracska, Péter Burkovics: The DNA-binding box of human SPARTAN contributes to the targeting of Pol η to DNA damage sites. DNA REPAIR 49 pp. 33-42., 10 p. (2017); MTMT: 3140736; IF: 4.461

*Shared first author

Conference

Presentation

Ágnes Tóth: Characterization of a novel G-quadruplex binding protein, 9th Central European Genome Stability and Dynamics Meeting, 2018 (Warsaw, Poland)

Ágnes Tóth, Gábor Harami, Karola Almási, Enikő Sajben-Nagy, Mihály Kovács, Péter Burkovics: Characterization of a novel G-quadruplex binding protein, Straub-days, 2018 (Szeged, Hungary)

Ágnes Tóth: The DNA-binding box of human SPARTAN contributes to the targeting of Polη to DNA damage sites, 7th Central European Genome Stability and Dynamics Meeting, 2016 (Zagreb, Croatia)

Poster

Ágnes Tóth, Péter Burkovics: Identifying new players in G-quadruplex unwinding and replication, Hungarian Molecular Life Sciences, 2017 (Eger, Hungary)

Ágnes Tóth, Lili Döme, Péter Burkovics, Lajos Haracska: Regulation of DNA damage tolerance pathway by human Spartan, 7th DNA Repair Workshop, 2016 (Smolenice, Slovakia)

Lili Döme, **Ágnes Tóth**, Kata Dudás, Lajos Haracska, Péter Burkovics: The regulatory function of ZBTB1 in the DNA Damage Tolerance pathway, Mechanisms of Recombination, 2016 (Alicante, Spain)