REVIEW

for the PhD thesis for the acquisition of the scientific and educational degree "doctor"

on the topic "KINETICS OF ACCUMULATION AND REMOVAL OF PROTEINS FROM THE REPLICATION FORK DURING ITS PAUSE AND RESTART",

by Teodora Krasimirova Dyankova-Danovska, full-time doctoral student at the Laboratory of Genome Stability, Institute of Molecular Biology "Acad. Rumen Tsanev", Bulgarian Academy of Sciences, in the scientific field 4.3. Biological Sciences (Molecular Biology) with scientific supervisor Assoc. Prof. Dr. Stoyno Stoynov

By Prof. Dr. Tanya Ivanova Topuzova-Hristova, Department of Cellular and Developmental Biology, Faculty of Biology, Sofia University "St. Kliment Ohridski"

Data on the PhD student and doctoral studies.

Teodora Krasimirova Dyankova-Danovska graduated with a Bachelor of Education degree in Molecular Biology from the Faculty of Biology of Plovdiv University "Paisii Hilendarski" in 2013 and a Master's degree in Genetic and Cellular Engineering from Sofia University "St. Kliment Ohridski" in 2015 with excellent results. Since 2014, she has been working as a biologist and later as an assistant in the section "Structure and Function of Chromatin" at the Institute of Molecular Biology, Bulgarian Academy of Sciences. Teodora Krasimirova Dyankova-Danovska was enrolled as a full-time doctoral student in 2016 in the Laboratory of Genomic Stability of the Institute of Molecular Biology of the Bulgarian Academy of Sciences, in the scientific field 4.3. Biological Sciences (Molecular Biology) with scientific supervisor Assoc. Prof. Dr. Stoyno Stoynov. Doctoral student Dyankova-Danovska has finished her study with the right to defend on time, as all requirements according to the Regulations for the implementation of the Law on the Development of the Academic Staff of the Republic of Bulgaria and the Regulations for the Development of the Academic Staff of the Institute of Molecular Biology "Acad. Rumen Tsanev" at the Bulgarian Academy of Sciences, section 4, were met and there were no violations. During the preparation of the dissertation for official defense, there was an interruption of the deadlines under the Law on the Development of the Academic Staff of the Republic of Bulgaria for the period of the state of emergency until 13.05.2020, on the basis of &24 of the transitional and final provisions of the Law on Measures and Actions during the State of Emergency of 13.03.2020. The minimal national requirements according to the Law on the Development of the Academic Staff in the Republic of Bulgaria, Promulgated by the State Gazette, No. 38 of 21 May 2010, amended by the State Gazette, No. 81 of 15 October 2010, amended by SG. No. 101 of December 28, 2010, amended SG. No. 68 of August 2, 2013, amended and supplemented SG. No. 30 of April 3, 2018,

amended SG. No. 17 of February 26, 2019, amended SG. No. 17 of February 25, 2020 are completed.

Dissertation data.

The topic of the dissertation "Kinetics of accumulation and removal of proteins from the replication fork during its arrest and restart" accurately reflects its content. The dissertation covers 108 pages, contains 35 complex figures, 6 schemes and 2 tables. The topic of the dissertation is relevant and essential for clarifying the role and behavior of the key proteins PCNA and RPA during replication arrest and restart.

The main parts of the dissertation follow the generally accepted plan for such work and include: Literature review – 23 pages, Goals and objectives – 1 page, Materials and methods – 10 pages, Results – 40 pages and Discussion – 7 and Conclusions and contributions – 1 page.

The literature review provides an overview of the data known in the literature on the processes of DNA replication, the proteins involved and their role. In separate points, attention is paid to the stages and regulation of replication. This part of the dissertation is written in understandable language, concisely and clearly, sufficiently motivating the choice of a scientific problem for further research.

The formulated goal and objectives logically follow from the review, and the materials and methods used are described correctly and clearly, in sufficient detail to be repeated. The aim of the dissertation is to study the kinetics of accumulation and removal of key proteins involved in replication during fork arrest and restart. 3 double HeLa Kyoto cell lines stably expressing the proteins RPA-EGFP, POLD2-EGFP, PAXIP-EGFP and PCNA-mCherry were used. All cell lines stably co-express mCherry-tagged mouse PCNA as a positive control and EGFP-tagged replication fork protein under the control of their endogenous regulatory sequences. To confirm the results in humans, a double PCNA cell line was additionally used, which expresses both human and mouse PCNA. All lines were created in the laboratory where the other studies related to the dissertation were conducted.

The set of methods includes both classical and modern methods (such as time-lapse microscopy, immunoblot and modified FRAP analysis). Basic theoretical principles that justify their use are also explained for the applied methods, accompanied by the necessary schemes. The experiments were performed precisely, and the obtained results were interpreted logically and with the necessary analyses. I have one question regarding the presented results, related to the immunoblot analysis: what was the quantification of the native and labeled proteins in the studied lines done?

The Results section presents the interface of the developed free access computer program CellTool, which unites all the necessary tools for analyzing the kinetics of the proteins involved in the process of DNA replication and repair, obtained from microscopic experiments, and the FRAP method. The results clearly show that the dynamics of the two proteins studied are directly dependent on the presence of replication stress and disruptions in the S-phase checkpoint for genomic integrity, caused by changes in the activity of key factors of DNA damage signaling. The kinetics of RPA under conditions of nucleotide depletion show that, regardless of the presence of

an active S-phase checkpoint, the gradual accumulation of RPA continues for up to 90 minutes and probably activates ATR, which arrests replication to prevent further accumulation of single-stranded DNA and depletion of RPA. Inhibition of ATM leads to the generation of single-stranded regions and the subsequent G2 phase, and from there to mitotic catastrophe. ATM inhibition has no effect on PCNA and RPA dynamics during fork stalling and restart in conditions of active ATR kinase, but co-inhibition of both ATM and ATR kinases reveals that ATM largely prevents the presence of single-stranded DNA after S phase when ATR is inhibited.

Most of the figures included in this section are complex, with a large number of time-lapse micrographs and graphical quantification of the two proteins in single cells, and give an idea of the large amount of work that has been done in these analyses. The results are well illustrated and explained, allowing for the drawing of 6 conclusions regarding the behavior of PCNA and PARP1 when replication is blocked by hydroxyurea and in the presence of ATM and ATR inhibitors. In a separate chapter, the results are discussed in comparison with what has been achieved by other research groups and the currently leading hypotheses for the participation of the studied proteins in the processes of replication arrest and restart. Some of the conclusions are formulated as a summary of the obtained results and need better refinement. In addition to the conclusions, two contributions of the dissertation work are also formulated, which I fully accept.

Scientific apparatus

234 sources are cited, which allow for a comprehensive review of scientific achievements on the topic. All sources are adequately selected and have a direct bearing on the research topic, in the range from 1981 to 2024, which shows the excellent awareness of the doctoral student. The citations are made in compliance with the established standards for citing scientific literature.

Abstract

The abstract contains 66 pages and fully reflects the content of the dissertation. The main results are correctly presented, illustrated with a total of 31 figures, which include graphs, color micrographs, immunoblot photos and schemes. The conclusions and contributions are correctly presented and correspond to those in the dissertation. Publications on the topic of the dissertation are also included, as well as a shortened list of cited literature, used mainly in the discussion of the results.

Publications

The main results of the dissertation work have been published in two articles in scientific journals with an impact factor and a quartile of Q1 and one manuscript, which is in the process of being reviewed. In two of the publications, the doctoral student is the first author (in one, the first place is shared), which reflects the significant contribution to their development. For the already published articles, separation protocols have been attached, reflecting the personal contribution of the doctoral student. Over 140 citations have been found for them in the scientific literature, which is an indicator of the high scientific value of these works. According to the requirements of the Academic Staff Development Low, the two already published articles carry a total of 50 points, which exceeds the required minimum of 30. In addition to these publications, Teodora Danovska

has attached a list of one additional publication and 10 participations in national and international conferences, at which she has popularized the results obtained in her dissertation research. This scientific and research activity characterizes Teodora Danovska as a well-established and active young scientist with excellent prospects for future development.

Scientific and applied scientific contributions

The contributions of the dissertation work include the development of a methodological approach for studying the dynamics of proteins in complex cellular processes and the data obtained from the application of this approach on the dynamics of RPA1, PCNA and POLD2 during the stalling and restarting of the replication fork under the conditions of an active and inhibited S-phase checkpoint. The first contribution is of a scientific and applied nature and refers to the new methodology with high temporal resolution for measuring and studying the dynamics of proteins involved in DNA replication during the stalling and restarting of the replication fork. The creation of a computer program with free access for analyzing microscopic images alone is a sufficiently large applied and methodological contribution, and the detailed manual for working with specified parameters and limits for the various protocols significantly facilitates its use. The second contribution is fundamental in terms of control of replication arrest and restart during the S-phase of the cell cycle.

Conclusion

In conclusion, a comprehensive study of the dynamics of RPA1, PCNA and POLD2 during replication fork arrest and restart under conditions of active and inhibited S-phase checkpoints is presented. The study was carried out using the developed open-access computer program CellTool, which combines all the necessary tools for analyzing the kinetics of proteins involved in the process of DNA replication and repair, obtained from microscopic experiments, and the FRAP method. The creation of an open-access computer program for analyzing microscopic images alone is a sufficiently large applied and methodological contribution, and the user-friendly interface and detailed manual for working with specified parameters and limits for different protocols significantly facilitate its implementation. New data were also obtained regarding the control of replication arrest and restart during the S-phase of the cell cycle.

Based on the submitted materials, I believe that the doctoral candidate fully meets the requirements of the Law on the State of the Republic of Bulgaria for the award of the scientific and educational degree "Doctor" and I recommend that Teodora Krasimirova Dyankova-Danovska be awarded the scientific and educational degree "Doctor" in the scientific field 4.3. Biological Sciences, scientific specialty Molecular Biology.

16.12.2024 Reviewer:

City of Sofia /Prof. Dr. Tanya Topuzova-Hristova/