

OPINION

By Assoc. prof. Galina Radeva, PhD

Roumen Tsanev Institute of Molecular Biology, BAS,

of PhD thesis of **Teodora Krasimirova Dyankova-Danovska** for the award of the educational and scientific degree "Doctor" (PhD) in the field of Higher education 4. Natural Sciences, Mathematics and Informatics, professional area 4.3 Biological sciences (Molecular Biology)

Author: Teodora Krasimirova Dyankova-Danovska

Title: Kinetics of Protein Accumulation and Removal from the Replication Fork during Its Stalling and Restart

Scientific supervisor: Assoc. prof. Stoyno Stoynov, PhD

General presentation of the materials under the procedure

By Order No. 1035/01.12.2024 of the Director of the Institute of Molecular Biology (IMB), I have been appointed as a member of the scientific jury for the defense of a dissertation for the acquisition of the educational and scientific degree "Doctor" in the professional field 4.3. "Biological Sciences," doctoral program "Molecular Biology." The dissertation, titled "„ Kinetics of Protein Accumulation and Removal from the Replication Fork during Its Stalling and Restart" is authored by Teodora Krasimirova Dyankova-Danovska, , a full-time doctoral student, supervised by Assoc. Prof. Dr. Stoyno Stoynov from IMB.

The set of materials submitted by Teodora Dyankova-Danovska on an electronic medium complies with the requirements of the Academic Staff Development Act of the Republic of Bulgaria (ASDA) and the Regulations for the Development of the Academic Staff of the Bulgarian Academy of Sciences (BAS) and IMB. It includes all documents required for the procedure.

Brief biographical data for the doctoral candidate

Teodora Dyankova-Danovska holds a Bachelor's degree in molecular biology from Plovdiv University Paisii Hilendarski (2013) and a Master's degree in Gene and cell engineering (2015), Sofia University "St. Kliment Ohridski," Faculty of Biology. From 2016, she was a full-time doctoral student in the "Molecular Biology" program at the Institute of Molecular Biology, Bulgarian Academy of Sciences (BAS). Danovska was a biologist in the Laboratory of Genome Stability at IMB, BAS from 2016 to March 2022.

Characteristics of the dissertation work

Relevance of the dissertation topic and feasibility of the set tasks

It is known that DNA replication in mammals is a complex and tightly regulated process. The mechanisms of DNA repair during replication and in its early stages, as well as the changes that occur in the replication fork under replication stress, are still not fully understood. The latter can lead to the stalling, slowing, or improper progression of replication. The kinetics of protein accumulation and removal from the replication fork plays a key role in ensuring an adequate cellular response and the restoration of replication. Cells have complex mechanisms for DNA protection and repair, but when these mechanisms are disrupted, genetic instability arises, increasing the risk of diseases. In this context, the group in the Laboratory of Genome Stability has a longstanding interest and significant results in elucidating this process.

The present dissertation is a continuation of these studies aimed at "investigating the kinetics of accumulation and removal of key proteins involved in replication during fork stalling and restart" using modern methods of spinning confocal microscopy and developed software for high-throughput image processing. A detailed understanding of the dynamics and quantitative determination of the proteins involved in DNA replication is essential for the development of anti-cancer therapies and strategies to prevent aging, which underscores the relevance of the developed topic.

Knowing the problem

The literature review provides an analysis of the current state of research on the issues addressed in the dissertation. It examines key processes such as DNA replication and termination, as well as the factors that can induce replication stress. Special attention is given to the cell cycle checkpoints, which play a crucial role in maintaining genomic stability. The manner in which the literature review is structured and the analytical presentation of the information show that Teodora Danovska knows the current state of the scientific problem being developed very well.

Research methods

A combination of modern methods of cellular and structural biology, mainly microscopic studies using a spinning disk confocal system, was selected for the implementation of the tasks. Methods of cell cultivation and treatment, immunoblotting, microirradiation of living cells were used. The CellTool software, developed by the team of the Laboratory of Genomic Stability, was used for the analysis of these experiments.

These methods correspond to the tasks, guaranteeing their execution.

Evaluation of the dissertation work

Danovska's dissertation is written on 107 pages and contains 41 figures, 2 tables and 234 cited references.

It is structured according to the generally accepted requirements for this type of works and contains the following sections: Introduction (3 p.); Literature review (23 p.s); Purpose and tasks (1 p.); Materials and methods (11 p.) Results (40 p.); Discussion (7 p.) Conclusions and contributions (1 p.) and List of references. A list of scientific publications and participation in scientific forums of the doctoral student are attached to the dissertation. The abstract is

written on 66 pages, and faithfully reflects the content of the dissertation and the contributions of the conducted research. The purpose of the dissertation is convincingly argued in the light of the literature review. To achieve this goal, five tasks have been formulated that correspond to the goal. Experimental work has been carried out on each task, the results of which are presented, very well illustrated, and discussed in the light of what is known about the relevant issues in the scientific literature.

At the end of the dissertation, 6 conclusions are drawn and 2 contributions of an original scientific and scientifically-applied are formulated, which faithfully and accurately present the main results of the conducted experimental studies.

The most significant scientific results and contributions from the research in the dissertation can be summarized as follows:

- A methodology has been developed for measuring and investigating the dynamics of proteins involved in DNA replication during fork stalling and restart with high temporal resolution in single living cells using spinning disk confocal microscopy;
- Using the CellTool software, the dynamics of the replication proteins RPA1, PCNA, and POLD2 were measured during fork stalling and restart under conditions of active and inhibited S-phase of the cell. The influence of the proteins ATR, PARP1, ATM, and MRE11 on the dynamics during stalling and restart was quantitatively assessed.

The achieved results contribute to elucidating the detailed mechanism of DNA replication in eukaryotes, specifically regarding the cellular response to replication stress, which can lead to genomic instability or even cell death. In the development of the CellTool software, Teodora Dyankova-Danovska participated in the design of the graphical user interface and image analysis protocols, as well as in the writing of the documentation and testing of the software on real experimental data.

On the topic of the dissertation, Teodora Dyankova-Danovska has presented three scientific publications in open access journals, ranked in quartile Q1, by the national minimum requirements for the educational and scientific degree "Doctor." In two of the publications, Dyankova-Danovska is the first author, alone or shared with Georgi Danovski. The publication in which she is the first author is under review and the manuscript is publicly available (DOI) as a preprint. The individual contributions and participation of the doctoral student in two of publications are clearly distinguished, with contributorship protocols provided for the authors' contributions in the specified papers. The presented articles meet and exceed the minimum national requirements for the educational and scientific degree "Doctor."

The doctoral candidate has participated in 10 national and international scientific forums.

Conclusion

The doctoral thesis of Teodora Dyankova-Danovska presents original results that contribute to elucidating the detailed mechanism of DNA replication in human cells, specifically regarding the cellular response to replication stress, which can lead to genomic instability or

even cell death. Precise studies on the kinetics of accumulation and removal of proteins involved in replication during fork stalling and restart have been carried out using advanced microscopy approaches. The dissertation demonstrates that Teodora Dyankova-Danovska has acquired the necessary level of scientific competence in the fields of molecular biology and bioinformatics, establishing herself as a highly qualified young researcher.

The dissertation meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the regulations for its application, including of the internal rules of IMB. Based on this, I give my positive evaluation and confidently recommend it to the esteemed members of the scientific jury to award the educational and scientific degree "Doctor" to Teodora Dyankova-Danovska in the professional field: 4.3. Biological Sciences, scientific specialty Molecular Biology.

18.12.2024

Prepared by:

/Assoc. prof. G. Radeva)

