



STATEMENT

By Assoc. Prof. Dr. Tanya Ivanova Topouzova-Hristova, Department of Cell and Developmental Biology,
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Regarding the dissertation thesis of Georgi Todorov Danovski for the acquisition of the scientific and educational degree "PhD" in direction 4.3. Biological Sciences (Molecular Biology) at the Laboratory "Genome Stability" with Scientific Director Assoc. Dr. Stoyno Stefanov Stoynov

Data on the doctoral student and the doctoral program:

Georgi Todorov Danovski graduated with a bachelor's degree in Molecular Biology at the Faculty of Biology of the Sofia University St Kliment Ohridski in 2013 and a master's degree in Biochemistry at the same Faculty in 2015. He was enrolled as a full-time doctoral student at the "Genome Stability" Laboratory at the Institute of Molecular Biology "Acad. R. Tsanev", BAS, with order No. 820 / 18.12.2015, with the topic of the dissertation "Mechanisms of spread of γ H2AX and MDC1 outside the zone of DNA damage" with scientific supervisor Assoc. Dr. Stoyno Stoinov. During the doctoral studies, the Danovski worked at the Institute of Molecular Biology as an assistant and biologist from 1.10.2015. All deadlines and criteria were met 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 on the Development of the Academic Staff of The Institute of Molecular Biology "Acad. Rumen Tsanev" at the Bulgarian Academy of Sciences, section 4 and no violations were committed.

Dissertation Data:

Topic. The thesis topic "Mechanisms of γ H2AX and MDC1 propagation beyond the DNA damage zone" reflects its content. The topic is particularly actual, with a view to establishing the precise mechanisms of marking and repairing damaged areas in genomic DNA, as well as the possibilities for mathematical modeling of protein kinetics in the complex biochemical repair network. The topic is part of the scientific issues actively developed in recent years by the "Genome Stability" Laboratory at the Institute of Molecular Biology "Acad. R. Tsanev", BAS, on which results are published in the most prestigious scientific journals in the field of cellular and molecular biology, such as Cell, Nature Communications, Molecular cell, Cell Division, PLoS ONE and others.

Main parts of the dissertation.

The literature review briefly presents the current knowledge about types of DNA damage and the repair systems that operate in eukaryotic cells, the causative agents of exogenous DNA damage and the role of γ H2AX and MDC1 in their marking and targeting of repair complexes. This part of the thesis is spread over 23 pages and is illustrated with 6 figures, the sources of which are duly cited.

The aim of the PhD thesis was to investigate the process of γ H2AX and MDC1 spreading outside the damage zone during the repair of complex DNA damage, as a consequence of the kinase activity of ATM, and to propose a mathematical model describing its mechanism. For the fulfillment of this goal, 4 tasks

have been formulated, which include both bio-informatics and molecular-biological methods at a modern level. Two of the tasks are related to the development of computer programs for the analysis of microscopic experiments with ultraviolet laser microirradiation and FRAP and for the calculation of biological reaction-diffusion models.

The methods and model systems used are adequate to the tasks and are at the most modern level. HeLa Kyoto cell lines were used, stably expressing proteins associated with DNA repair processes and associated with GFP or mCherry, which were analyzed by applying the FRAP method combined with immunofluorescence determination of γ H2AX around the focus of damage. A mathematical model was made and the images were analyzed using the CellTool computer program created by the PhD student Danovski. The analyzes of the microscopic images and the mathematical formulas used for signal normalization, FRAP analysis, and reaction-diffusion equation modeling are detailed in 14 pages.

In the Results part, the developed open-access computer program CellTool is presented, which combines all the necessary tools for analyzing the kinetics of proteins involved in the DNA repair process obtained from microscopic experiments and the FRAP method. The program was validated with a cell line expressing two fluorescently tagged proteins – involved in DNA repair and leaving sites of damage. The protocol was also adapted for FRAP analysis, tracking MDC1 and ATM in the area of complex damage in different models (eg, in the presence or absence of RAD21).

Five conclusions have been formulated that summarize the achieved results of the individual tasks and five contributions of a theoretical-applied and methodological nature. The creation of an open-access computer program for microscopic image analysis alone is a large enough applied and methodological contribution, and the addition of standardized protocols for FRAP analyses, a mathematical apparatus, and a program for modeling biological processes by reaction-diffusion equation and theoretical model describing distribution of γ H2AX and MDC1 as a consequence of ATM diffusion makes the work extremely valuable.

Scientific apparatus. 274 sources are cited, including articles with the participation of the doctoral student in connection with the dissertation work. The abundance of sources used shows a deep knowledge of the developed subject.

Author's summary of dissertation. The summary contains 52 pages and essentially reflects the content of the dissertation work. The parts included are Introduction, Aim and objectives, Materials and methods, Results, Discussion, Conclusions and Contributions.

Publications. The doctoral student has submitted a list of a total of three publications on the topic of the dissertation - two articles published in periodicals and one that is in the process of peer review. The two articles are in Q1 quartile journals and carry a total of 50 points, which exceeds the minimum number of 30 points for strand 4.3. Biological sciences, according to Appendix 1 of the ZRASRB. In one of the published and peer-reviewed articles, the doctoral student is the first author, which is an indicator of the substantial contribution to the experimental data and obtained results included in the respective publications. By the time the dissertation defense procedure was launched, the mentioned two publications received a total of over 100 citations in the scientific literature (120 according to WoS and 198 in Google Scholar), which is a good certificate of the significance and relevance of the obtained results.

Conclusion. The thesis submitted for opinion is dedicated to a fundamental area of biomedical research, namely the elucidation of the molecular and control mechanisms of complex DNA damage repair. As part of the planned tasks, two computer programs were created and validated for the analysis of microscopic images and a theoretical model explaining the obtained data on the behavior of two of the proteins involved in the processes of detection and repair of complex DNA damage. The requirements of ZRASRB for the scientific and educational degree "doctor" have been met, and I give my positive assessment regarding the awarding of the scientific and educational degree "PhD" in direction 4.3. Biological Sciences (Molecular Biology) to Georgi Todorov Danovski.

31.05.2024

Statement by:

City Sofia

/ Assoc. Dr. Tanya Topouzova-Hristova/