SCIENTIFIC OPINION

From Assoc. Prof. Galina Simeonova Radeva, PhD,

Roumen Tsanev Institute of Molecular Biology, BAS

Concerning: the doctoral thesis of Georgi Todorov Danovski titled "Mechanisms of γH2AX and MDC1 spread beyond the DNA damage site"

General presentation of the procedure and the candidate

I was elected as a member of the Scientific jury by ordinance № 54-ОВ/26.03.24 (Director of Roumen Tsanev Institute of Molecular Biology, BAS) and Protocol 1/12.04.24 to prepare a scientific opinion for doctoral thesis entitled: "Mechanisms of γH2AX and MDC1 spread beyond the DNA damage site" for the award of the educational and scientific degree "Doctor" (PhD) in Professional field 4.3 Biological sciences, scientific specialty Molecular Biology". The author of the thesis is Georgi Danovski with supervisor Assoc. Prof. Stoino Stoinov, PhD.

The doctoral thesis presented by Georgi Danovski is written on 107 pages and contains 23 figures and two tables, and 274 cited literature sources. In terms of volume, structure, and design, the thesis fully meets the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB) and the relevant Regulations for its implementation (including those of the BAS and IMB) as well as the set of digital materials. The dissertation abstract, written on 52 pages, properly reflects the content of the dissertation and the contributions of the research included in the dissertation and meets the requirements of the above-mentioned documents.

Actuality of the subject, aim and research tasks

The submitted thesis for scientific opinion is focused on a fundamental study of the mechanisms of DNA repair and the function of the proteins involved in this process, their dynamics and their spatial distribution. It is known that the mechanisms of DNA repair represent a complex biochemical network involving more than 300 proteins that participate in complex biochemical interactions, forming interconnected pathways. The study of DNA repair requires in-depth knowledge not only of the functions of the proteins and their interactions, the changes in their regulatory regions that affect the repair factors but also of their dynamics and spatial distribution in the cell for the efficient process and to maintain the genetic stability of the cells. To elucidate this process, the thesis aims to study the process of the distribution of γH2AX and MDC1 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.

It is an extremely current and markedly interdisciplinary scientific field, uniting various parts of molecular biology, bioinformatics and mathematical modeling of biological processes with multiple applications.

PhD student knowledge of the topic

The literature review is focused on DNA damage and the cellular processes that repair this damage. The literature review is structured and the analytical presentation of the information shows that Georgi Danovski is familiar with the problem in detail. The aim of the dissertation stems from the need to precisely measure the kinetics of accumulation and removal to sites of damage of a large number of proteins involved in the DNA repair process. To reach this goal, 4 specific, interrelated tasks have been formulated, which lead to creating a quantitative model describing the accumulation and distribution in the space of ATM and MDC1 in the presence of complex DNA damage. The experiments performed and the quantitative models analyzed confirm the doctoral student's hypothesis that the basis of the spread of MDC1 (mediator of DNA damage checkpoint protein 1) is the diffusion of activated ATM (Ataxia-telangiectasia mutated kinase).

Methodology of the study

In the methodological part - section "Materials and methods" the experimental procedures and the performed data processing are described in detail. In the course of working on the dissertation, the PhD student became familiar with the methods of cell and molecular biology, microscopic techniques for working with living and fixed cells, and analysis of microscopic images. Mathematical modeling of the accumulation and distribution of DNA repair proteins at sites of complex damage was performed. The above-mentioned methods are well combined in the conducted research and contribute to clarifying the mechanisms of repair of the most serious damages - DNA double-strand breaks. It should be noted that the PhD student used a wide range of methods, they are very well illustrated and described, which allows their correct reproduction.

Characterization and evaluation of the dissertation and contributions

The presented results - section "Results" in the dissertation are in full agreement with the set goal and the correctly defined tasks for implementation. The achieved results show the relevance of the developed topic. The presentation of the results is combined with a competent discussion at all stages of the development of the problem, based on appropriate references, which shows a deep knowledge of the PhD student in the field of molecular biology and bioinformatics.

Based on the achieved results, five conclusions were drawn and five original contributions were summarized, with which the set goal was achieved. I accept the wording of the contributions and want to emphasize their importance in a theoretical and scientific-applied aspect, as follows:

Fundamental contributions are related to the detailed characterization of the spatiotemporal distribution of ATM and MDC1 at sites of complex DNA damage induced by UV laser microirradiation, which reveals the mechanism of γH2AX distribution as a key step of the DNA repair process.

Scientifically-applied contributions include the creation of the computational platform for the analysis of microscopic images, named CellTool, which allows the measurement of the kinetics of the accumulation of proteins involved in the DNA repair process. CellTool has a user-friendly graphical interface that provides fast, easy and accurate image analysis, as well as standardized protocols for fast and qualitative analysis of UV laser microirradiation and fluorescence recovery after photobleaching (FRAP) experiments. The program is convenient for the analysis of a wide range of microscopic data, allowing the precise tracking and quantification of changes in fluorescence intensity.

The results of the dissertation are published in two scientific publications in open-access journals *International Journal of Molecular Sciences* and *Molecular Cell*, with quartile Q1, which is a confirmation of the high level of research. In one of the publications, Georgi Danovski is the first author, which is a testimony to his leading participation and contribution. He has presented the results of the dissertation work, mainly with reports presented at ten international and national scientific forums.

Conclusion

The doctoral thesis contains scientific and scientifically applied results that respond to all requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB) and the relevant Regulations for its implementation (including those of the BAS and IMB). The dissertation shows that Georgi Danovski has acquired the necessary level of scientific competence in the field of molecular biology, in particular in bioinformatics, where he has obtained interesting and valuable scientific results. Based on the above, I give my positive assessment and strongly recommend to the respected members of the scientific jury to award the educational and scientific degree **"Doctor"** to **Georgi Danovski** in professional direction 4.3. Biological Sciences, scientific specialty "Molecular Biology".

30.05.2024

Signature

/Assoc. prof. G. Radeva/