OPINION

By Assoc. prof. Galina Radeva, PhD

Roumen Tsanev Institite of Molecular Biology, BAS,

of dissertation of prof. Stefan Ivanov Dimitrov for the award of the scientific degree "Doctor of Science " in Professional area 4.3 Biological sciences (Molecular Biology)

Title: „ Choromatin -from Structure to Function"

Author: prof. Stefan Ivanov Dimitrov, PhD

The present review of Prof. Stefan Dimitrov's dissertation entitled "Chromatin – from Structure to Function" is based on Order No. 209-OB/06.12.2024 issued by the Director of the IMB for the appointment of a Scientific Jury. The set of materials submitted by prof. Stefan Dimitrov 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 for professional area 4.3 Biological sciences (Molecular Biology).

Prof. Dimitrov has presented an original dissertation dedicated to the structure and function of chromatin and the role of epigenetic factors in maintaining nuclear homeostasis in the cell, which has been his primary scientific focus for 40 years. His interest in this field began during his tenure at IMB. Since 1996, he has successfully developed this research direction, initially as co-Head of the "Chromatin Structure and Function" team at Ecole Normale Supérieure de Lyon, Lyon, and later as a head of the "Chromatin and Epigenetics" laboratory at the Institute Albert Bonniot, Grenoble, where was First Class Director of Research, National Center of Scientific Research (CNRS). At present, he is Emeritus Director of Research, at the National Center of Scientific Research (CNRS) and supervisor of ERA Chair Project AEGIS-IMB at the Roumen Tsanev Institute of Molecular Biology, BAS.

The specific objectives of the dissertation are: to resolve the structure of the H1-associated nucleosome, to determine the three-dimensional organization of the chromatin fiber, and to analyze how the structure of nucleosomes containing a specific histone variant defines their function.

The research spans a significant period, with the first cited article related to the dissertation published in 1999. The dissertation work involves collaborations with teams from international research institutions.

Prof. Dimitrov's dissertation consists of 310 pages and includes the following sections: Acknowledgments, Introduction, Literature review, Objectives, List of publications, used for the dissertation, Results, Summary and Discussion, and References. More than 70% of the dissertation is dedicated to the results. This section includes 15 original published scientific papers and one manuscript submitted for publication.

The research strategy is based on a multidisciplinary approach and incorporates biochemical, molecular biology, and biophysical methods, as well as microscopy techniques, including cryo-electron microscopy, X-ray crystallography, and high-resolution hydroxyl radical footprinting for DNA-protein interactions, along with cell biology methods. As model systems, the studies utilize nucleosomes reconstituted with histone variants H2A.Z, H2A.Bbd, macroH2A, and CENP-A, as well as an H1-associated hexanucleosome, together with *in vitro* studies of H1-associated 6-, 12-, and 24-nucleosome arrays.

The original contributions related to the dissertation can be summarized as follows:

The crystal structure of the H1-associated nucleosome and the cryo-EM structure of the chromatin fiber have been determined. The results show that the binding of H1 to the nucleosome, as well as the interaction with various H1 mutants, leads to a more compact and rigid conformation of the nucleosome and induces changes in the dynamic flexibility of the linker DNA.

The 3D structure of the chromatin fiber and the 3D structure of mitotic chromosomes have been determined based on the H1-associated 6-nucleosome unit. It has been shown that nucleosomes are arranged through identical interfaces, adopting a flat zigzag structure with a packing density that is half that of the twisted 30 nm fiber. These findings shed light on the plastic organization of chromatin, which can switch between different conformations in response to specific epigenetic strategies, thereby influencing its function.

It has been established that the organization of the nucleosome, is associated with various histone variants—macroH2A, H2A.Bbd, H2A.Z, and CENP-A—and their remodeling play a key role in the proper functioning of the nucleus. These histone variants modulate chromatin structure, and the resulting changes affect the organization of the cell's genome.

Prof. Dimitrov's dissertation makes a fundamental contribution to the global scientific literature in the field of chromatin structure and function.

The results presented in this dissertation have been included in 15 scientific publications, published in top-ranked international journals (quartile Q1) in the field of structural and cell biology, such as Molecular Cell, EMBO Journal, Nucleic Acids Research (NAR), Nature, PNAS, and others. The high scientometric indicators of these publications, with a total impact factor of 198, attest to the high scientific quality of the conducted research. In most of them prof. Dimitrov is a corresponding author, which highlights his leading role in chromatin research over the years. Prof. Dimitrov's reputation as an internationally recognized scientist is further confirmed by

2,335 citations (Scopus and WoS) from foreign authors of works related to the dissertation. Prof. Dimitrov's scientometric indicators significantly exceed the minimum required points for the degree of "Doctor of Sciences," as specified in the ZRASB for the scientific field and professional domain 4.3. Biological Sciences.

The abstract is 40 pages long and accurately reflects the content of the dissertation and the contributions of the research.

Conclusion

Prof. Stefan Dimitrov's dissertation presents original results from an extensive fundamental study on the structure and function of chromatin, obtained through the effective application of a combination of cutting-edge methods in cellular and structural biology. The research conducted is both relevant and significant, receiving broad recognition in citations from the international scientific community and establishing the author as a leading global authority in the field of molecular biology. Based on the above, I express my positive assessment of the presented dissertation and strongly support the awarding of the academic degree "Doctor of Sciences" to Prof. Dr. Stefan Dimitrov in the professional field 4.3. Biological Sciences (Molecular Biology).

09.02.2025

Signed by

/Assoc. Prof. Galina Radeva, PhD/