OPINION

Submitted by Assoc. Prof. Dr. Dimitar Borisov Iliev, Institute of Molecular Biology - BAS

On the dissertation for the award of the scientific degree "**Doctor of Sciences**" in professional field 4.3. Biological Sciences; Scientific specialty Molecular Biology

Author: Prof. Dr. STEFAN DIMITROV - Institute of Molecular Biology - BAS
Topic: CHROMATIN – FROM STRUCTURE TO FUNCTION

1. **General Presentation of the Procedure and the Candidate**
   By Order № 110-OB/28.06.2024 of the Director of the Institute of Molecular Biology "Rumen Tsanev" at the Bulgarian Academy of Sciences (IMB-BAS), I was appointed as a member of the scientific jury in connection with the procedure for obtaining the scientific degree "Doctor of Sciences" in professional field 4.3. Biological Sciences, scientific specialty Molecular Biology.

   At the subsequent hybrid meeting of the scientific jury on 16.12.2024, I was tasked with preparing an opinion.

   The dissertation covers research carried out over a 15-year period and includes 15 scientific papers and one manuscript sent for publication. The dissertation is structured as follows:

   2. Acknowledgements
   3. Introduction
   4. Objectives
   5. List of publications used for the dissertation
   6. Results
   7. Summary and Discussion

The materials presented by Prof. Dimitrov are in accordance with the Rules for the Development of the Academic Staff of IMB-BAS and meet the criteria of IMB-BAS for obtaining the scientific degree "Doctor of Sciences."

2. **Biographical Information**

   • Prof. Stefan Dimitrov obtained a "Master" degree in Physics from Sofia University "St. Kliment Ohridski" in 1976.
   • In 1982, the candidate defended a dissertation in molecular biology for the acquisition of PhD degree at IMB-BAS and the Institute of Molecular Biology in Moscow.
   • Between 1983 and 1996, Prof. Dimitrov held various positions at:

- IMB-BAS
- Swiss Institute for Experimental Cancer Research, Switzerland
- The Cancer Research Center, Laval University, Canada
- National Institutes of Health (NIH) in Bethesda, USA

• From 1996 to 2017, Prof. Dimitrov worked as a scientific director at the National Centre for Scientific Research (CNRS), head of a laboratory at the Institute for Advanced Biosciences, Grenoble, France, and co-leading a lab at Ecole Normale Supérieure de Lyon, France.
• From 2018 to 2023, the candidate served as the scientific director of the Izmir Biomedicine and Genome Center (IBG) in Izmir, Turkey, and was the head of the Epigenetics and Chromatin Biology laboratory at IBG.
• Since 2023, Prof. Dimitrov has been appointed as ERA Chair in the project "Advanced Epigenetics Studies to Increase the Research and Innovation Capacity of the Roumen Tsanev Institute of Molecular Biology of the Bulgarian Academy of Sciences (AEGIS-IMB)."

Prof. Dimitrov's rich and exceptionally fruitful scientific career has been awarded numerous prizes and honors in Bulgaria and abroad, including being an honorary member of the Turkish Academy of Sciences (TUBA), a foreign member of the National Academy of Sciences in India (NASI), and a foreign member of the Bulgarian Academy of Sciences.

Prof. Dimitrov's indicators are outstanding:
- **157 scientific papers**
- **> 12,000 citations**
- **Google Scholar h-index – 59**
- **Google Scholar i-index – 101**

Equally impressive is his activity as a supervisor of over 90 students and postdocs, many of whom continue to develop successful careers in science and industry.

Furthermore, Prof. Dimitrov is one of the founders of the "Laboratoire Joliot Curie" at Ecole Normale Supérieure de Lyon (ENS) – one of the most prestigious universities in France. He is also a co-founder of the Izmir Biomedicine and Genome Center (iBG-izmir) – the largest research center for molecular and cell biology in Turkey. The candidate also plays a key role in the development of epigenetic research in India.

2. **Relevance of the Topic**

The studies of the structure and function of genetic material are essential for unraveling fundamental physiological and pathophysiological processes in living organisms. The revolutionary discovery of the DNA structure by Francis Crick and James Watson in 1953 was followed by over 40 years of research, leading to the discovery of the structure of the nucleosome particle – the second fundamental discovery that stimulated both modern biology and medical research. Prof. Dimitrov's work led to resolving the next two major mysteries in the field of structural epigenetics, which had challenged the epigenetic and chromatin community for decades: the structure of the nucleosome bound to histone H1, and the 3D

organization of the chromatin fiber. This highlights the relevance and significance of the topic of the current dissertation.

3. Characteristics and Evaluation of the Dissertation and Contributions

The introduction of the dissertation is well-focused and provides a clear overview of the relevance and importance of the research, serving as a solid justification for the objectives and tasks of the dissertation.

The results are presented in a non-traditional but well-grounded manner. The first pages of the articles, containing the abstracts and some figures, have been used to illustrate the research achievements. This presentation allows for a clear understanding of the research level while avoiding unnecessary repetition of already published data.

A strongly interdisciplinary approach has been applied in the work on this dissertation. A broad range of structural techniques were employed, including X-ray diffraction and cryo-electron microscopy, molecular dynamics simulations, physicochemical, biochemical, and cell biology methods, epigenetic and bioinformatics approaches examining the entire genome, combined with genetic techniques in mice. This unique technical scenario enables the determination of the binding of linker histone H1 to the nucleosome and shows how its binding aids the organization of the chromatin filament into a 30 nm chromatin fiber. As a result of this highly complex approach, the intimate structural details of these molecular-level objects are convincingly illustrated. Additionally, experiments on the elasticity of mitotic chromosomes are expected to shed light on their internal 3D organization.
The summary and discussion are presented in a concise but well-structured manner, allowing insight into the essence of the results, the conclusions drawn, and their significance.

The main scientific contributions of the works included in the dissertation are:

1. Clarification of the structure of the nucleosome bound to H1, which represents a breakthrough in the structural chromatin epigenetics.
2. Confirmation of the double-stranded organization of short nucleosomal arrays in vitro and a hypothesis regarding the mechanism by which they condense into a 30-nanometer fiber.
3. Information on how the cell uses the incorporation of major histone variants (H2A.Z, macroH2A, H2A.Bbd, and CENP-A) to modulate chromatin structure and how these changes affect genome organization and enable nuclear events, or how changes in genome conformation become function.

4. Evaluation of the publications and the personal contribution of the candidate

Fifteen of the papers upon which the dissertation is based have been published in leading scientific journals with high impact factors, which is undeniable evidence of the quality of the work and the obtained results. The candidate is the corresponding author in some of these publications (including in *Nature* with an IF=64.8), which reflects Prof. Dimitrov's leading role in this research.

CONCLUSION

As a member of the scientific jury, I firmly express my support for awarding the scientific degree "Doctor of Sciences" to Prof. Dr. Stefan Dimitrov. The candidate is a world-renowned scientist with an impressive professional biography. The dissertation contains original contributions in terms of the scientific results obtained, which shed light on fundamental biological structures and mechanisms, and fully meets the requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Application of ZRASRB, and the Regulations for the Application of ZRASRB at BAS, as well as the specific requirements of the IMB-BAS Regulations for the Application of ZRASRB in professional field 4.3. Biological Sciences, scientific specialty Molecular Biology.

Date: 10.02.2025

Prepared by:

.....

(Assoc. Prof. Dr. Dimitar Iliev)