



## REVIEW

of a dissertation for the award of the scientific degree  
"Doctor of Science" in the Professional direction 4.3. Biological Sciences, Scientific  
(Molecular Biology)

**Topic of the dissertation:** "Chromatin - from structure to function"

**Author:** Prof. Dr. Stefan Ivanov Dimitrov

**Reviewer:** Acad. Prof. Dr. Roumen Georgiev Pankov, member of the scientific jury, appointed by order of the Director of the Institute of Molecular Biology "Acad. Roumen Tsanev" at the Bulgarian Academy of Sciences 209-OB/06.12.2024.

### Brief biographical details of the applicant

Prof. Stefan Dimitrov graduated from the Faculty of Physics of Sofia University "St. Kliment Ohridski" in 1976 with a degree in "Solid State Physics". In 1982 he became a doctor in molecular biology at the Institutes of Molecular Biology (IMB) in Sofia and Moscow. The same year he started working as a research associate at IMB Sofia, and five years later he was elected a senior research associate. Between 1990 and 1992 he worked as a visiting scientist and professor in Switzerland and Canada. From 1993 to 1996 he was a visiting scientist in the laboratory of the world-renowned specialist in the field of epigenetics A. P. Wolf from the National Institutes of Health (NIH) of the USA.

Since 1996 he works in France, at the Ecole Normale Supérieure de Lyon, and at the Albert Boniot Institute in Grenoble, where he leads laboratories focused on epigenetic issues. He has been a professor at the National Center for Scientific Research (CNRS) of France for more than 20 years, and since 2017 he has been an honorary professor at the same institution. Currently, Prof. Dimitrov is a European Research Area (ERA) Chair Holder, with a project implemented at the Institute of Molecular Biology at the Bulgarian Academy of Sciences.

Prof. Dimitrov is one of the founders of the interdisciplinary institute "Joliot Curie Laboratory" at the Ecole Normale Supérieure de Lyon, and is a member and later Chairman of the Scientific Advisory Board of the largest Center for Cell and Molecular Biology in Turkey - the Izmir Biomedical and Genomic Center (iBG-Izmir). During the period 2020-2023 he is the Scientific Director of IBG-Izmir. For more than 20 years, he has supported the establishment and operation of laboratories in India engaged in research in the field of epigenetics.

Prof. Dimitrov has been awarded numerous awards, including Foreign Member of the Bulgarian Academy of Sciences and the National Academy of Sciences of India (NASI). He is also a full (honorary) member of the Turkish Academy of Sciences (TUBA).

### Relevance of the dissertation topic

Knowledge of the structure and three-dimensional organization of the H1-bound nucleosome and chromatin filaments is crucial for understanding fundamental biological processes. Histone H1 plays a key role in chromatin compaction, influencing gene expression, DNA replication and repair. It helps stabilize nucleosome interactions and facilitates the formation

of higher-order chromatin structures, which are fundamental for the efficient packaging of DNA in the nucleus. This in turn affects gene expression and epigenetics, as chromatin accessibility determines transcriptional activity. Understanding how different H1 variants and post-translational modifications affect chromatin structure is vital for decoding gene regulatory mechanisms and has significant medical and therapeutic implications. Deep knowledge of chromatin structure has a direct connection not only with the progress of modern medical and biological fields such as regenerative medicine and synthetic biology, but also with practice, such as the development of new therapies for diseases such as Alzheimer's and Huntington's and various types of cancer. Without a doubt, the elucidation of chromatin structure and functions is a contemporary issue that has not only fundamental but also significant practical applications.

### **Structure, goals and methodological approach of the dissertation**

For his dissertation, Prof. Stefan Dimitrov used the format "collection of scientific publications accompanied by a comprehensive literature review and connecting discussion", referred to in item 2.2. of the Regulations for the implementation of the IMB's ZARSB. The dissertation contains 311 pages and consists of a literature review, designated as Introduction (50 pages), Goals (2 pages), Results - presented in the form of 16 full-text scientific articles, Summary and Discussion (5 pages) and Literature, including 178 sources.

The introduction of the dissertation is very well focused, clearly written and in its first part "Chromatin" describes the available data for the first two levels of chromatin organization - the nucleosome and chromatin filaments. Particular attention is paid to the interaction of the linker histone H1 with both the nucleosome and the chromatin filament and the resulting conformational transitions and final configurations of these objects. The author has critically analyzed the experimental data and proposed models for the structure of the H1-bound nucleosome and the 30 nm chromatin filament. His analysis shows that despite hundreds of articles devoted to these questions, they still remain open and represent the two main challenges for researchers studying the structure and epigenetic role of chromatin. The second part of the Introduction focuses on the role of histone variants - the main epigenetic factors that the cell uses to change the epigenetic landscape of chromatin and to control basic nuclear processes and nuclear homeostasis. Prof. Dimitrov presents in depth and summarizes extremely professionally the published data on the organization and proposed functions of the major histone variants with an emphasis on macroH2A, H2A.Z, H2A.Bbd and CENP-A. This comprehensive and insightful presentation of the existing research provides a clear understanding of the challenges in the study of nucleosomes with histone variants and prepares the reader to understand the significant contribution of the results presented in the dissertation.

The presented study aims to answer two of the most important and debatable questions in the epigenetic field: 1) What is the structure of the nucleosome, connected to H1 and the 3D organization of the chromatin fibril and 2) How the major histone variants macroH2A, H2A.Bbd, H2A.Z and CENP-A, allow the proper functioning of the nucleus by modulating the organization of nucleosomes. A wide range of the most cutting-edge interdisciplinary methodological approaches has been used to solve the tasks set. High-resolution structural and imaging techniques (Single-Base Resolution Mapping, Cryo-Electron Microscopy, X-ray Crystallography, Atomic Force Microscopy), biophysical and computational modeling approaches (Nanoscale Molecular Modeling, Fluorescence Resonance Energy Transfer, Optical Tweezers & Chromosome Elasticity Assays), biochemical and functional genomic techniques (Chromatin Immunoprecipitation, In Vitro and in vivo Nucleosome Assembly and Remodeling Assays, DNA Damage and Repair



Assays), genetic and epigenetic manipulations (RNAi Knockdowns, Histone Mutagenesis Studies), etc. were used. This high-tech and complex approach undoubtedly guarantees high precision, reproducibility and reliability of the obtained data.

### **Analysis of experimental results, conclusions and contributions**

The main part in terms of importance and volume in the dissertation is the “Results” section, presented, as noted, in the form of 16 scientific articles. The experimental data obtained are presented in detail, supported by abundant and high-quality illustrative material, discussed in depth and have undergone rigorous peer review by international experts in the field. Without being exhaustive, I will note the results that, in my opinion, represent the most significant scientific achievements.

In my opinion, the most significant contribution of the dissertation is the determination of both the structure of the H1-bound nucleosome and the 3D organization of the chromatin filament. This was achieved thanks to the use of the most powerful structural methods, including X-ray diffraction and cryo-electron microscopy, combined with a cohort of approaches from molecular biology, biochemistry and physical chemistry. The team of Prof. Dimitrov showed that H1 binding induces a more compact and rigid conformation in the nucleosome. The H1 globular domain interacts with the DNA core of the dyad and with both DNA linkers, while the C-terminal domain associates primarily with a single DNA linker. These findings reveal that H1 imparts a strong degree of asymmetry to the nucleosome, which likely influences the assembly and architecture of higher-order structures. These results were further confirmed in the studies of Prof. Dimitrov's team on the 3D organization of the chromatin filament and its mode of condensation upon binding of the linker histone H1. To study the 3D organization of the chromatin filament, Prof. Dimitrov used hexanucleosome arrays reconstituted on tandem 6x601 DNA using purified recombinant histones. Since 601 DNA has a very strong signal for nucleosome positioning, the samples are of high homogeneity, which allows to obtain crystals with a resolution of 9.7 Å, as well as to determine the cryo-electron microscopic structure of these arrays. The data reveal that the nucleosomes are organized in a flat double-sided helix with the same phase of arrangement. It should be noted that increasing the ionic environment leads to folding into a more compact helix, which is probably accompanied by small changes in the way the globular domain of histone H1 interacts with the nucleosome dyad. In addition, using elasticity measurements, Prof. Dimitrov has analyzed the folding of chromatin fibers in mitotic chromosomes and proposed an intriguing model for their structure.

The results described above are summarized in the first five articles and have been published in some of the best journals, including *Mol. Cell*, *PNAS* and *J. Cell Biol.* Importantly, the in vitro structures of both the H1-bound nucleosome and the chromatin filament have recently been shown, using cryo-electron microscopy, to also exist in vivo. Thus, Prof. Dimitrov and his collaborators have solved two major problems of structural epigenetics that have been the subject of the scientific community for more than forty years. I think this represents an outstanding contribution that can be compared even with the determination of the structure of DNA and the nucleosome, published in 1953 and 1997, respectively.

How does the chromatin filament unfold to allow DNA binding factors to gain access to the underlying DNA sequences and extract the various genetic information necessary for the survival of the cell? This important question is the subject of the second part of the dissertation, which investigates how the assembly of nucleosomes with histone variants, which have a structure different from that of conventional nucleosomes, affects fundamental nuclear events, such as



transcription, chromatin remodeling, and centromere assembly (these issues are investigated in the remaining 11 articles). The first and pioneering contribution of Prof. Dimitrov's team on this topic is focused on the most deviant histone variant, namely macroH2A. It has been found that macroH2A interferes with the binding of transcription factors and SWI/SNF nucleosome remodeling. In contrast, another histone variant, H2A.Bbd, promotes the transcription of nucleosome arrays containing H2A.Bbd nucleosomes, and H2A.Z is indirectly involved in transcriptional regulation. The latter result was obtained from in vivo experiments using conditional knock-out H2A.Z mice. Furthermore, H2A.Z is involved in both mitosis and senescence and is very likely to be a major guardian of genome integrity. These functions of H2A.Z are determined by its specific localization in the genome, which is achieved through the interaction of the special chaperones for H2A.Z identified by Prof. Dimitrov - ANP32e (responsible for removal) and YL1 (responsible for incorporation). The author also participated in the identification and study of the properties of HJURP - the chaperone for the histone variant CENP-A. HJURP-mediated deposition of CENP-A on centromeric DNA leads to the assembly of CENP-A nucleosomes with an intriguing structure, which, as shown by the author's team, exhibit extremely unusual properties necessary for the proper course of mitosis.

Prof. Dimitrov's work on nucleosomes with histone variants has been summarized in a series of articles published in prestigious journals (*Nature*, *Mol. Cell*, *Nature Structural and Molecular Biology*) and has received a very wide international response. They have an innovative nature and have been used as a platform for numerous subsequent studies by various laboratories around the world. Prof. Dimitrov is considered one of the best specialists in histone variants.

#### **Compliance of the submitted dissertation with the ZRASB and the Regulations for the Development of the Academic Staff of the IMB "Acad. Roumen Tsanev".**

The dissertation work and the abstract are prepared in accordance with the ZRASB and the Regulations for the Development of the Academic Staff of the Institute of Molecular Biology "Acad. Roumen Tsanev" at the Bulgarian Academy of Sciences. The submitted scientific production fully meets the profile of the announced competition.

For the current procedure, Prof. Stefan Dimitrov has submitted 15 published articles and one proposed for publishing. All publications have been printed in some of the most prestigious scientific journals in the field of medical and biological sciences, such as *Nature*, *Nature Struct. Mol. Biol.*, *Nucleic Acids Res.*, *Molecular Cell*, *The EMBO J*, *J. Cell. Biol.*, etc. All articles are in first quartile (Q1) journals, and their total impact factor is 213.4. The high quality of the research conducted can also be illustrated by the very wide international response that they have received - the noted citations of these publications are 2335. It should be pointed out that in 13 of the articles (81%) Prof. Dimitrov is a corresponding author, which is indisputable evidence of his leading role in the research conducted.

The report on the fulfillment by Prof. Dimitrov of the minimum national requirements under Art. 2b of the ZRASRB for the scientific field 4. Natural sciences, mathematics and informatics professional field: 4.3 Biological sciences, shows the following results: group A - 50 points; group B - 100 points; by indicator G - 375 points with a required 100 points, and by indicator D(citations) - 4670 points with a minimum of 100 points. Thus, with a required minimum of 350 points in total for the scientific degree "Doctor of Sciences", according to the PPZRASRB, Prof. Stefan Dimitrov formed 5195 points, which many times exceeds the national requirements necessary for this scientific degree.

The abstract is prepared in accordance with the requirements and reflects all the main results of the dissertation.

I have one suggestion regarding the design of the presented dissertation. I believe that readers would benefit if the most important contributions from the research conducted were formulated briefly and included in the dissertation.

**Conclusion:**

The dissertation "Chromatin - from structure to function", presented by Prof. Dimitrov is a focused research of exceptionally high scientific caliber, which meets the requirements for a dissertation for obtaining the scientific degree "Doctor of Sciences". It is the result of many years of purposeful research work that led to the clarification of two of the most important problems of the structural epigenetics of chromatin. A series of pioneering studies have been conducted, published in some of the most prestigious scientific journals. The results of these studies have had a wide resonance in the scientific community and have earned Prof. Dimitrov an extremely high reputation as a specialist in the field of chromatin biology and epigenetics. All of the above gives me reason to give my positive assessment and to confidently recommend to the esteemed members of the Scientific Jury to award Prof. Dr. Stefan Ivanov Dimitrov the scientific degree "Doctor of Sciences".

07.02.2025

Reviewer:



/Acad. Prof. DSc Roumen Pankov/