REVIEW

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On the dissertation of Prof. Stefan Dimitrov for the award of the scientific degree "Doctor of Science"

The work submitted to me for review on the topic "Chromatin from structure to function" consists of four chapters: introduction, goals, results and summary and discussion. In the chapter "Introduction and background" the author presents the accumulated data on the packaging of DNA in the eukaryotic nucleus and the organization of the genome. Particular attention is paid to the structure of the main nucleosome particle and the folding of the chromatin filament in the chromatin fiber. The organization of the histone octamer and its interactions with nucleosome DNA are analyzed in detail and the "behavior" of the NH2-histone terminal tails is discussed. The available data on the structural properties of the linker histone H1 and its function are described, and the open questions about the interaction of H1 with nucleosome DNA, one of the main problems of structural epigenetics, are clearly formulated. The data accumulated in recent years on the 3D structure of the chromatin filament are described and analyzed, presenting the models that have become established in the literature. The second part of the Introduction focuses on how the presence of histone variants gives new structural properties to the nucleosome and how this determines specific functions. In particular, the structure of the nucleosome containing one of the main histone variants, namely macroH2A, H2A.Z, H2A.Bbd and CENP-A, is examined. I would like to note that the author has managed to present a very large volume of published results on the topic in a very synthesized and clear way, analyzing the achievements in the light of epigenetics. The goals of the dissertation are clear and very well defined and logically derived from the facts presented in the literature review. The first main goal is aimed at the two main problems in structural epigenetics, namely the structure of the H1-bound nucleosome and the

3D organization of chromatin fibers, problems in which the epigenetic community has shown great interest for more than four decades. The second group of goals is focused on histone variants, their mode of incorporation into chromatin and their role in nuclear homeostasis. In the Results section, the author has used an unconventional, but largely accepted by the international community, way to present his experimental data. The results are presented as a collection of some of the author's publications that directly address the goals of the dissertation. The results of the interactions and binding of histone H1 to the nucleosome and the chromatin filament are described in 4 articles (#1-4 of "Publications used for the dissertation"). Using a wide range of structural, biophysical and biochemical approaches, a detailed mapping of linker histone H1-DNA interactions at single DNA base resolution is described. After data processing, both the crystal and cryo-EM structures of the H1-bound nucleosome were determined. The data show that H1 binding induces a more compact and rigid conformation of 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 predominantly with a single DNA linker. As a model system for determining the structure of chromatin fibers, Dimitrov's team used hexanucleosomes with very clearly positioned nucleosomes. X-ray diffraction data unambiguously show that the crystal structure of the hexanucleosome can be described as a two-start helix with identical nucleosome stacking interfaces. Hydroxyl radical footprinting, biophysical, cryo-EM and cross-linking data confirm the crystal structure and reveal that a small change in the ionic environment shifts the conformational pattern towards a more compact, twisted form. How chromatin fibers fold in mitotic chromosomes is the next focus of Dr. Dimitrov, who by measuring their elasticity has proposed an original model for their structure (abstract #5). In summary, this first part of the dissertation resolves the two main open questions of structural epigenetics. It is important to note that recent cryo-microscopy data confirmed these results in vivo. The original results obtained by Dimitrov et al.

can be considered a significant breakthrough in the field of epigenetics. The second part of Dr. Dimitrov's dissertation analyzes how the incorporation of a number of histone variants, the main epigenetic factors, affects both the structure and function of chromatin (articles 6-15). This part has a markedly interdisciplinary character and uses different approaches: structural biology, physical chemistry, biochemistry, molecular and cell biology and epigenetic methods, combined with mouse genetics.

Taken together, the data identify some specific structural features of macroH2A, H2A.Bbd, H2A.Z and CENP-A nucleosomes and illustrate how these features determine their specific functions. The histone macroH2A nucleosome was found to interfere with transcription factor binding and SWI/SNF nucleosome remodeling (article #6). Dimitrov's team also analyzed the structure and functionality of the nucleosome with the histone variant H2A.Bbd by studying SWI/SNF remodeling and p300-dependent transcription of H2A.Bbd nucleosome arrays. It was found that, unlike macroH2A nucleosomes, H2A.Bbd nucleosomes show lower stability both in vitro and in vivo, which favors transcription of H2A.Bbd chromatin (articles #7-9). The nucleosome CENP-A is an epigenetic marker of centromeres, its formation is carried out with the help of HJURP, which serves as a chaperone, and has flexible ends. The original results were published in article #10. Intriguingly, the flexibility of these ends is necessary for proper mitotic function, since in vivo hardening of nucleosome ends leads to serious problems and disorders in the process of mitosis (article #11).

In the last section of the second part, the effects of the histone variant H2A.Z are analyzed. Prof. Dimitrov and colleagues have identified the first mammals for H2A.Z. They prove that the protein ANP32E is able to remove H2A.Z from the nucleosome and replace it with conventional H2A (article #12), while the reverse process involves YL1, which deposits H2A. Z in chromatin (article #13). The last three articles shed light on the function of H2A.Z in vivo using different techniques and H2A.Z knockout mice (articles #14-16). The

results clearly show that H2A.Z is one of the main factors in maintaining genome integrity and nuclear homeostasis. Depletion of H2A.Z in basal epidermal stem cells leads to activation of cytosolic DNA sensor pathways, which subsequently leads to the development of an inflammatory process. These facts present in a completely new light H2A.Z-dependent interrelationships between epigenetic, immune and inflammatory processes.

Taken together, Dr. Dimitrov's results on histone variants are completely original and have paved the way for a large number of studies on the function of these proteins, both in vitro and in vivo. They have been widely cited in the literature and have gained wide international recognition.

Conclusion: The dissertation submitted to me for review is an extremely voluminous and in-depth work on the role of histone variants. The author not only traces the structural changes in the nucleosome, but also analyzes cellular mechanisms in depth, reporting specific dependencies in the processes of chromatin remodeling, mitosis, inflammation, etc. I believe that the original results achieved, the characterized structural models and the hypotheses expressed represent an extremely valuable contribution to the development of epigenetics. The application of innovative approaches in research can serve as a basis for future development of new topics and directions related to the structure and functions of chromatin and epigenetic control.

I most strongly propose that Prof. Stefan Diomitrov be awarded the scientific degree of "Doctor of Sciences".