

## **OPINION**

**by Assoc. Prof. Galina Radeva, PhD**

**Roumen Tsanev Institute of Molecular Biology, BAS,**

of PhD thesis of Aleksandar Sergeev Ategin for the award of the educational and scientific degree "Doctor" (PhD) in the field of Higher education 4. Natural Sciences, Mathematics and Informatics, professional area 4.3 Biological Sciences (Molecular Biology)

Author: Aleksandar Sergeev Ategin

Title: „Study of the dynamics of processes in living cells using modern microscopic approaches"

Scientific supervisor: Assoc. Prof. Marina Nedelcheva-Veleva, PhD

Co-supervised: Assoc. Prof. Stoyno Stoynov, PhD

### **General presentation of the materials under the procedure**

By Order No. 132-OB/03.09.2024 of the Director of the Institute of Molecular Biology (IMB), I have been appointed as a member of the scientific jury for the defense of a dissertation for the acquisition of the educational and scientific degree "Doctor" in the professional field 4.3. "Biological Sciences," doctoral program "Molecular Biology." The dissertation, titled "Study of the dynamics of processes in living cells using modern microscopic approaches" is authored by Aleksander Sergeev Ategin, a full-time doctoral student, supervised by Assoc. Prof. Dr. Marina Nedelcheva-Veleva and co-supervised by Assoc. Prof. Dr. Stoyno Stoynov from IMB.

The set of materials submitted by Aleksander Ategin 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.

### **Brief biographical data for the doctoral candidate**

Aleksander Ategin graduated from Sofia University "St. Kliment Ohridski," Faculty of Biology, earning a Bachelor's degree in Biotechnology (2014) and a Master's degree in Gene and cell engineering (2016). From 2016 to 2024, he was a full-time doctoral student in the "Molecular Biology" doctoral program at the Institute of Molecular Biology, Bulgarian Academy of Sciences (BAS). Since 2016, he has been working as a biologist in the Genome Stability Laboratory at IMB., BAS. He possesses excellent proficiency in English.

### **Characteristics of the dissertation work**

#### **1. Relevance of the dissertation topic and feasibility of the set tasks**

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to significant morbidity and mortality on a global scale. This beta-coronavirus continues to evolve, with global health implications, while scientists strive to learn more to limit its spread, evolution, and consequences. The development and implementation of vaccines and antiviral therapeutics against SARS-CoV-2 have significantly reduced the local to global impact and burden of COVID-19.

Despite substantial efforts by scientists worldwide, there is still no specific and definitive therapy for the disease, as SARS-CoV-2 rapidly evolves into variants with increased transmissibility and enhanced ability to evade the immune system. There is a pressing need to intensify efforts to understand this novel virus, its variants, and the short- and long-term effects of COVID-19 on human health.

Of particular interest are SARS-CoV-2 and its Omicron variant, which are the focus of precise studies on the virus's life cycle. There is still a lack of information on one of its key stages, specifically the mechanism by which the virus enters the host cell.

The aim of the dissertation submitted for my review is to shed light on this issue, specifically: to investigate the dynamics of the processes accompanying the entry of SARS-CoV-2 virus-like particles into host cells, and to study the dynamics of the levels and intracellular distribution of proteins involved in DNA replication during the cell cycle.

Given the above, I believe that the topic of the dissertation is relevant and significant in a scientific and applied sense. Six tasks have been set for implementation, which I consider to be purposeful and their implementation will lead to the achievement of the goal.

## **2. Knowing the problem**

The literature review offers an in-depth analysis of the current state of research on the thesis issue: etymology of the SARS-CoV-2 virus, its genetics and structure, life cycle, replication, existing and possible treatment therapies, current therapeutics and treatment strategies, etc.

Also, the literature review describes DNA replication and the proteins responsible for this process. How the literature review is structured and the analytical presentation of the information shows that Aleksander Ategin knows the current state of the scientific problem being developed very well.

## **3. Research methods**

A combination of modern methods of cellular and structural biology, mainly microscopic studies using a spinning disk confocal system, was selected for the implementation of the tasks. Methods of transfection and work with cell cultures were used. Various software developed in the "Laboratory of Genomic Stability" such as CellTool, SPARTACUSS, etc., were used for the tracking and analysis of the images after the microscopic observations. These methods correspond to the tasks, guaranteeing their execution.

## **4. Evaluation of the dissertation work**

Atemin's dissertation is written on 128 pages and contains 24 figures, 1 table and 247 cited references.

It is structured according to the generally accepted requirements for this type of works and contains the following sections: Introduction (3 pages); Literature review (36 pages); Purpose and tasks (1 page); Materials and methods (7 pages) Results (56 pages); Discussion (6 p.) Conclusions and contributions (2 p.) and List of references. A list of scientific publications and participation in scientific forums of the doctoral student is attached to the dissertation. The abstract is written on 74 pages and faithfully reflects the content of the dissertation and the contributions of the conducted research. The purpose of the dissertation is convincingly argued in the light of the literature review. To achieve this goal, six tasks have been formulated that correspond to the goal.

Experimental work has been carried out on each task, the results of which are presented, very well illustrated, and discussed in light of what is known about the relevant issues in the scientific literature.

The most significant scientific results and contributions from the research in the dissertation can be summarized as follows:

**The fundamental contributions** pertain to the processes of the cell cycle during the entry of SARS-CoV-2 virus-like particles, the Omicron variant, and a SARS-CoV-2 variant with a deletion at the furin cleavage site of the S protein, known as internalization. These include their binding to the cell membrane, changes in morphology and acidification of the environment, release of the viral nucleocapsid, and the active microtubule-dependent movement of particles. The precise expression profile and distribution of five proteins—RIF1, MCM6, ORC1, CLASPIN, and PCNA—are involved in the replication process during the cell cycle. These results unveil fundamental aspects of viral entry into the cell and could be valuable for the development of virus-like particles as carriers for drug delivery, vaccines, or gene therapy.

**Scientifically applied contributions** pertain to the development of 1) Detailed methodologies for visualizing and accurately measuring changes in the speed and intensity of labeled SARS-CoV-2 virus-like particles, as well as measuring the levels and distribution of specific proteins during the cell cycle using fluorescence microscopy and 2) The creation of web-based databases, COVIDynamics and DNArepairK Database, aimed at supporting research and visualization of the internalization of SARS-CoV-2 virus-like particles, as well as the dynamics of proteins involved in DNA repair. The database thus created serves as a tool for explaining various molecular and biological processes and as an instrument useful for both the scientific and non-scientific communities.

On the topic of the dissertation, Alexander Atemin has presented three scientific publications in the journals *Viruses*, *Biomedicines*, *IJMS*, ranked in quartile Q1. The total number of points for Indicator G is 75 (with a minimum requirement of 30 points), following the national minimum requirements for the educational and scientific degree "Doctor." In two of the publications, Aleksander Atemin is the first author, which testifies to his leading role and contribution to the research conducted and its presentation. The doctoral candidate has participated in four national and seven international scientific forums.

My personal impression of Aleksander Ategin is that of an ambitious and well-prepared young scientist in the field of molecular biology, possessing skills in conducting scientific research, analyzing and summarizing data, as well as presenting the results of his work in a talented manner. I recommend that he pay more attention to the technical formatting of the dissertation, avoid the excessive use of English terms, and employ more precise stylistic expressions.

**Conclusion:**

The research conducted in Aleksander Ategin's dissertation represents a high scientific standard, offering novel contributions to science. The findings significantly enhance our understanding of a fundamental process of the cell cycle: the entry of viruses into host cells. Moreover, the study establishes the expression and distribution of licensed proteins involved in DNA replication, utilizing advanced microscopic techniques.

The PhD student has mastered a wide variety of experimental and theoretical methods, as well as the ability to present his research to the scientific community.

The dissertation meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the regulations for its application, including the internal requirements of IMB. All this gives me a reason to give a positive assessment of the conducted research, the results of which are presented in detail in the dissertation work. I strongly recommend the esteemed scientific jury to award the educational and scientific degree "Doctor" to Aleksandar Sergeev Ategin in the professional field: 4.3. Biological Sciences, scientific specialty Molecular Biology.

18.11.2024

Prepared by:

/Assoc. Prof. G. Radeva, PhD/