REVIEW

By Prof. Dr. Tanya Ivanova Topuzova-Hristova, Department of Cellular and Developmental Biology, Faculty of Biology, Sofia University "St. Kliment Ohridski"

Regarding the dissertation thesis of Alexander Sergeev Atemin, a full-time doctoral student at the Laboratory of Genomic Stability, Institute of Molecular Biology "Acad. Rumen Tsanev", Bulgarian Academy of Sciences, for the acquisition of the scientific and educational degree "PhD" in direction 4.3. Biological Sciences (Molecular Biology) with scientific supervisor Assoc. Prof. Dr. Marina Nedelcheva-Veleva and scientific consultant Assoc. Prof. Dr. Stoyno Stoynov

on the topic **"Studying the dynamics of processes in living cells through modern microscopic approaches"**

Data regarding the doctoral student and the doctoral program.

Alexander Sergeev Atemin has a bachelor's degree in Biotechnology at the Faculty of Biology of Sofia University "St. Kliment Ohridski" in 2014 and a master's degree in Genetic and Cellular Engineering at the same University in 2016 with excellent results. Since the same year, he has been appointed as a biologist in the "Structure and Function of Chromatin" section at the Institute of Molecular Biology, BAS and has been enrolled as a full-time doctoral student in 2016 in the Laboratory of Genomic Stability of the Institute of Molecular Biology at the Bulgarian Academy of Sciences, in the scientific direction 4.3. Biological Sciences (Molecular Biology) with scientific supervisor Assoc. Prof. Dr. Marina Nedelcheva-Veleva. Doctoral student Atemin has completed the necessary courses, passed the relevant exams and has been discharged with the right to defend his thesis on time, having met all the requirements according to the Regulations for the Implementation of the Law on the Development of the Academic Staff of the Republic of Bulgaria and the Regulations for the Development of the Academic Staff of the Institute of Molecular Biology "Acad. Rumen Tsanev" at the Bulgarian Academy of Sciences, Section 4, and has not committed any violations. During the preparation of the dissertation for official defense, there is an interruption of the deadlines under the ZRASRB for the period of the state of emergency until 13.05.2020, on the basis of &24 of the transitional and final provisions of the Law on Measures and Actions during the State of Emergency of 13.03.2020. The approbation of the dissertation was held on 22.07.2024. All those present united around the opinion that the work is distributable and meets the requirements according to the ZRASRB, and the procedure for open defense was launched. The minimum national requirements according to the Law on the Development of the Academic Staff in the Republic of Bulgaria, Promulgated by the State Gazette, No. 38 of May 21, 2010, amended by the State Gazette, No. 81 of October 15, 2010, amended by the State Gazette, were also met. No. 101 of

December 28, 2010, amended by SG No. 68 of August 2, 2013, amended and supplemented by SG No. 30 of April 3, 2018, amended by SG No. 17 of February 26, 2019, amended by SG No. 17 of February 25, 2020.

Dissertation data.

The topic of the dissertation "Studying the dynamics of processes in living cells using modern microscopic approaches" accurately reflects its content. The dissertation covers 136 pages, contains 24 figures, 1 table and 2 schemes. The use of modern microscopic techniques in combination with processing the obtained data with appropriate software products and creating databases accessible to a wide range of researchers is the basis of the topic of the dissertation work and is extremely relevant for the clarification of various cellular processes of fundamental and applied importance.

The main parts of the dissertation follow the generally accepted plan for such work and include: Literature review – 36 pages, Goals and objectives – 1 page, Materials and methods – 6 pages, Results – 30 pages and Discussion – 6 and Conclusions and contributions – 2 pages in total.

The literature review provides an overview of the processes of entry of SARS-CoV and SARS-CoV-2 viral particles, their replication and assembly into cells, with an emphasis on the therapeutic potential of viral proteins – membrane, nucleocapsid and others, if used as targets. Since the process being monitored is the entry of virus-like model particles itself, it would be more appropriate to focus on the variants of endocytosis and the proteins involved in this process. Instead, the various medications, vaccines and therapies used in practice are described in unnecessary detail, but no connection is made to the main topic of the dissertation. However, as an advantage, I can point out that the dynamics of viral particles and possible variants of their entry into cells have been tracked, which leads logically to the Goals and objectives set in the next section. As a complementary task with the idea of optimizing the approach for tracking the dynamics of various complexes in the cell, the proteins ORC1, MCM6, CLASPIN, RIF1, PCNA related to replication and the cell cycle are also considered. In the introduction and literature review, there are semantic and typographical errors made inadvertently, as well as the use of unnecessary foreign words, which should be cleaned up before the final publication of the work.

The formulated goal and tasks logically follow from the review, and the materials and methods used are described correctly and clearly, in sufficient detail to be repeated. The aim of the dissertation work is to study the dynamics of the processes accompanying the entry of SARS-CoV-2 virus-like particles into host cells and the dynamics of the levels and distribution in the cell of proteins involved in DNA replication during the cell cycle.

Three cell lines (A549, VeroE6, U2OS) and their variants with increased expression of ACE2 and TMPRSS2 were used to monitor viral internalization, as well as HeLa Kyoto, which stably expresses RIF1, ORC1, PCNA, MCM6, Claspin, labeled with EGFP. Tracking of virus-like particle entry was performed in cells transfected with fluorescently labeled dynamin, ACE2 and mNeonGreen, and endosomal trafficking was monitored by organelle-specific staining with CellLight™ Early Endosomes-GFP, BacMam 2.0 and LysoTracker. The culture media, plasmid vectors, antibodies and chemicals are described in detail, including catalog numbers, and the

set of methods includes both classical and modern methods (TEM, transfection of human cells with fluorescently labeled proteins, in vivo observations and documentation of labeled proteins by confocal microscopy and subsequent processing with a set of software solutions in the Python program, as well as in Fiji, which are collectively called SPARTACUSS (Single-Particle Tracking Analysis in Cells Using Software Solutions). Detailed explanations are given for the applied methods, which would be useful when repeating the approach. The experiments were performed precisely, and the obtained results were interpreted logically and with the necessary mathematical and statistical analyses. The obtained results clearly show that the viral particles were successfully tracked during their adhesion to the cell membranes, as well as during their entry into the cells, and this process was compared with the dynamics of proteins and organelles key to endocytosis. A huge amount of work has been done in terms of individual tracking of virus-like particles outside and inside individual cells, collecting complex images that can then be further processed with appropriate software, measuring fluorescence intensity and pH of intracellular structures and comparing them with moving virus-like particles, etc. The results are well illustrated and explained, allowing for the derivation of 7 clearly formulated conclusions regarding the speed of SARS-CoV-2 virus-like particles after entering the host cell, the mechanism of entry into the cell and the release of virus particles from endosomes. The last (eighth) conclusion is regarding the dynamics of replication-related proteins during the cell cycle, but it is not formulated specifically enough, but too generally.

In addition to the conclusions, five contributions of the dissertation work are formulated, which I fully accept. For me, the most significant contributions of this dissertation work are the two created information databases with microscopic images and videos, which are freely accessible and would be of exceptional help to researchers of replication processes, cell cycle control, as well as to virologists studying SARS-CoV variants. No less important are the methodological contributions - two detailed methodologies have been compiled, allowing the visualization and accurate measurement of changes in the speed and intensity of labeled virus-like particles; the in vivo measurement of the levels of a certain fluorescently labeled protein, as well as its distribution in the cell during the cell cycle, as well as a protocol for the discrete determination of the phases of the mitotic cycle, as well as early S, mid S and late S phases of the cell cycle, by fluorescently labeled PCNA, measured after applying the Sobel operator, which will certainly be used in future studies.

Scientific apparatus

247 sources are cited, which allow for a comprehensive review of scientific achievements on the topic. All sources are adequately selected and have a direct bearing on the research topic, which shows the excellent awareness of the doctoral student. The citations are made in compliance with the established standards for citing scientific literature.

Abstract

The abstract contains 75 pages and fully reflects the content of the dissertation work. The main results are correctly presented, illustrated with a total of 24 figures, which include graphs and color micrographs. The conclusions are presented correctly. Publications on the topic of the

dissertation are also included, as well as a shortened list of cited literature used mainly in the discussion of the results.

Publications

The main results of the dissertation work have been published in three publications in scientific journals with an impact factor and a quartile (two in Q1 and one in Q2). In the publications with quartile 1, the doctoral student is the first author, which reflects the significant contribution to their development. According to the requirements of the Academic Staff Development Act, the three publications carry a total of 70 points, which exceeds the required minimum of 30 more than twice. In addition to these publications, Alexander Atemin has attached a list of three additional publications, participation in 11 national and international conferences, at which he popularized the results obtained in his dissertation research and other scientific works. This significant publication activity characterizes Alexander Atemin as a well-established and active young scientist with excellent prospects for future development.

Scientific and applied science contributions

The contributions of this dissertation are undeniable and significant for both fundamental science and applied science. Web-based databases COVIDynamics and DNArepairK Database have been created, which are useful for studying the internalization pathways of SARS-CoV-2 virus-like particles, as well as the dynamics of proteins associated with DNA replication and repair. Methodologies have been developed for tracking and analyzing the entry and movement of fluorescently labeled particles into the cell, which would help in virological studies, but also in the development of new nanoscale drug carriers in pharmacology; and also for quantifying the dynamics of fluorescently labeled regulatory proteins involved in the licensing and conduct of DNA replication.

The fundamental science contributions are related to five key processes associated with the entry of virus-like particles into two different cell lines and with the dynamics of key replication proteins throughout the cell cycle. I accept the formulated contributions of the dissertation.

Conclusion

In conclusion, a complex study of virus-like particles during their adhesion to cell membranes, as well as their entry into cells from two different cell lines, is presented, and this process is compared with the dynamics of proteins and organelles key to endocytosis. A huge amount of work has been carried out in terms of individual tracking of virus-like particles outside and inside individual cells, collection of complex images, which can then be further processed with appropriate software. Tracking of proteins key to DNA replication throughout the entire cell cycle has also been carried out, and a protocol has been created for determining the phases based on the dynamics of the studied proteins. Two web-based databases and new methodological approaches to studying the dynamics of cellular processes have been created.

Based on the submitted materials, I believe that the doctoral candidate fully meets the requirements of the Bulgarian Academy of Sciences for awarding the scientific and educational degree "Doctor" and I recommend that Alexander Atemin be awarded the scientific and

educational degree "Doctor" in the scientific field 4.3. Biological Sciences, scientific specialty Molecular Biology.

11.11.2024 Reviewer:

City of Sofia /Prof. Dr. Tanya Topuzova-Hristova/