OPINION

by Professor Dr. Rumiana Dimitrova Tzoneva, Institute of Biophysics and Biomedical Engineering - BAS

Regarding the competition for the position of "Associate Professor" in Field 4: Natural Sciences, Mathematics, and Informatics, Professional Field 4.3: Biological Sciences, Scientific Specialty "Molecular Biology, " as announced in the State Gazette, Issue 104 from 2024, for the needs of the Laboratory of Genomic Stability, IMB - BAS.

Senior Assistant Professor Dr. Radoslav Alexandrov Alexandrov is the only candidate in the announced competition. The documents submitted by the candidate for the academic position "Associate Professor" have been prepared following the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its application at the Institute of Molecular Biology "Acad. Rumen Tsanev (IMB) at the Bulgarian Academy of Sciences.

Candidate's biographical data

Radoslav Alexandrov graduated with a bachelor's degree in molecular biology and a master's in biochemistry from Sofia University "St. Kliment Ohridski", Faculty of Biology, in 2012 and 2014, respectively. In 2013, while pursuing a master's degree in Biochemistry, he participated in a scientific exchange under the Erasmus program at the PHYMOTS laboratory (Laboratoire Physiologie Moléculaire du Transport des Sucres chez les Végétaux) at the University of Poitiers, France, where he worked on his thesis under the supervision of Prof. Dr. Rositsa Atanasova, focusing on elucidating the mechanisms of sugar signaling in plants.

From 2014 to 2015, he worked as a specialist biologist in the Laboratory of Genomic Stability at IMB-BAS. Between 2015 and 2019, he was enrolled as a full-time doctoral student in the laboratory under the supervision of Assoc. Prof. Dr. Stoyno Stoynov. On 19.12.2018, he successfully defended his dissertation on the topic "Dynamics and sequence of binding of proteins involved in DNA repair." A significant part of the results included in the dissertation was published in the prestigious journal *Molecular Cell* and reveals the kinetics of accumulation and removal of 70 proteins involved in DNA repair mechanisms in living cells. Thanks to the systematic study conducted, new essential aspects of the coordination of DNA repair pathways were revealed, and a significant achievement is the first-time discovery of the influence of PARP1/2 on the normal chronology of the repair process. Between 2019 and 2022, Radoslav Alexandrov was part of the Laboratory of Genomic Stability at the Institute of Molecular Biology-BAS as a researcher under the National Program "Young Scientists and Postdoctoral Fellows." Since February 2022, he has been an assistant professor at the Laboratory. During this period, the candidate's scientific work has primarily focused on uncovering the mechanism of action of PARP inhibitors and other drugs in living cells, targeting DNA repair systems, and developing an experimental approach. The main findings of these studies were published in a scientific article in *Cell Reports* (PMID: 38758646), of which Radoslav Alexandrov is the lead author.

Publication activity

In this competition, Senior Asst. Prof. Dr. Radoslav Alexandrov participated with a total of 14 publications outside the publications included in the doctoral dissertation, of which 13 were published in refereed journals in Scopus/Web of Science with an impressive total impact factor of 113.227, which were cited 534 times (Scopus, 29.04.2025). Among them are articles published in the prestigious journals Cell and Cell Reports. Of these, 11 (eleven) issues with

Q1, 1 (one) issue with Q2, and 1 (one) issue with Q3. In one of the articles, Dr. Alexandrov is the first and last author in two articles. Consequently, in 2018, 2021, and 2024, publications in which the candidate is the first or last author were selected as the best scientific publications of the Institute of Molecular Biology.
Dr. Alexandrov has published 15 scientific papers in refereed journals indexed in Scopus and Web of Science, with a total impact factor of 140.065 and an h-index of 7, according to SCOPUS.

Scientific projects
Dr. Radoslav Alexandrov has participated in 11 national and one international scientific projects and has been the leader of 2 national and one international project.
In 2024, Dr. Radoslav Alexandrov won a prestigious international project funded by the Swiss National Science Foundation (SNSF) under the Promotion of Young Scientists in Central and Eastern Europe (PROMYS) program. The project, which lasted five years and had a total value of 1,300,000 leva, was on the topic of "Deciphering DNA damage response dynamics in living cells."

Graduate student support
Senior Asst. Dr. Radoslav Alexandrov supervises four graduates: three bachelor's (Faculty of Biology, Sofia University) and one master's (Maastricht University).

Participation in scientific events
Dr. Radoslav Alexandrov has participated in 14 national and international conferences, at which he presented six poster presentations and eight reports, including invited reports at the Genome Architecture and Function Workshop in Sofia in 2024 and the iPoLS Annual Meeting—Physics of Living Systems in Houston, USA, in 2018.

Awards
In 2019, the Bulgarian Academy of Sciences awarded Dr. Radoslav Alexandrov the "Marin Drinov" Award for a young scientist and the award for the best scientific publication by a young scientist in the competition on the occasion of the Bulgarian Academy of Sciences' 150th anniversary.
In 2019, Dr. Radoslav Alexandrov was awarded the "Rumen Tsanev" award for young scientist by the Institute of Molecular Biology.

Research activity
Senior Assistant Professor Dr. Radoslav Alexandrov's research activity is in molecular and cellular biology and biochemistry. It is mainly related to studies of the dynamics of DNA repair and replication in living cells, elucidation of the etiology of chronic rhinosinusitis, establishment of metabolic differences between embryogenic and non-embryogenic plant cells, as well as research on the mechanism and properties of the neurotoxin vipoxin, isolated from *Vipera ammodytes meridionalis.*

Main Contributions

I Research on the dynamics of DNA repair in living cells

- A unified model has been proposed for studying the mechanism of action of PARP inhibitors and quantifying their effectiveness. The model introduces and measures three precise experimental parameters that accurately quantify the dynamics and activity of the

PARP1 protein at sites of DNA damage in living cells. The new approach can be successfully applied to study the mechanism of action of other drug molecules targeting DNA repair systems [Publication B4.1].

- A new function of the PARP1 protein has been identified, namely its participation in the ligation of broken DNA ends by keeping them close to each other in living cells. This newly discovered function of PARP1, dependent on its catalytic activity and the accumulation of the PAR-binding protein FUS, impacts the stabilization of the resulting PARP1-DNA condensates at the break site [Publication B4.2].
- A unique open-access database has been created: DNARepairK (http://dnarepair.bas.bg/index.php/dnarepairk/). It contains the kinetics of accumulation and removal of 70 proteins involved in DNA repair at sites of complex DNA damage in living cells. [Publication B4.3].
- A unique open-access software for processing microscopic images has been created: CellTool (https://dnarepair.bas.bg/software/CellTool). The CellTool software can analyze and mathematically model the dynamics of accumulation, removal, and exchange of proteins from DNA repair systems at sites of DNA damage in living cells. The many filters, segmentation and tracking algorithms, and mathematical models integrated into it allow for much faster analysis of such data compared to other image processing software [Publication B4.4].
- The mechanism of the spread of ATM-dependent phosphorylation of histone H2AX and the accumulation of the MDC1 protein in regions significantly larger than the site of DNA damage has been revealed through the combined application of microscopic observation, dynamic simulations of living cells, and mathematical modeling [Publication D7.2].

II. Study of the dynamics of DNA replication in living cells

- The dynamics of the proteins PCNA and RPA1 at replication sites (replication foci) in control human cancer cells and those with induced replication stress with hydroxyurea have been revealed. Using this approach, the role of the kinases ATM and ATR in the dynamics of replication in human cells has also been studied [Publication G7.1].

- The role of the protein Dia2 in *Saccharomyces cerevisiae* in cell cycle progression and the size and shape of cells in control cells and those under replication stress conditions has been revealed [Publication G7.5].

III. Study of the etiology of chronic rhinosinusitis

- A strong correlation was revealed between the formation of a bacterial biofilm on the mucous membrane of the nasal cavity and the development of chronic rhinosinusitis by the methods of confocal microscopy and quantitative PCR analysis. [Publication G7.4].

IV. Study of metabolic differences between embryogenic and non-embryogenic plant cells

- Significant differences in proliferation potential, enzyme activity, metabolic pathways, and oxygen consumption levels have been established in embryogenic and non-embryogenic plant cell cultures with a common genetic origin. [Publication G7.7].

V. Study of the mechanism and properties of the neurotoxin vipoxin, isolated from *Vipera ammodytes meridionalis*

- Through biochemical approaches, key amino acid residues involved in the active center of the enzymatic subunit of the toxin vipoxin sPLA2, isolated from the venom of *Vipera ammodytes meridionalis*, which have a direct catalytic role, as well as amino acid residues necessary for substrate binding and stability of the active center have been identified [Publication G7.10].
- A set of scFv clones was obtained that neutralize the toxic sPLA2 subunit of lipoxin and can be used for in-depth studies of the toxin's physiological effects [Publication G7.9].
- The cytotoxic effects of sPLA2 on a panel of cell lines were established, as well as the impact of sPLA2 on the integrity of the cell membrane [Publication G7.8].

Conclusion: From the presented scientific works of Senior Assistant Professor Dr. Radoslav Alexandrov, it is evident that the overall scientific production of the candidate satisfies and significantly exceeds the minimum requirements of the Law on Academic Development in the Republic of Bulgaria and the criteria for acquiring the scientific title "Associate Professor" at the Institute of Molecular Biology - Bulgarian Academy of Sciences for the professional field "Biological Sciences", scientific specialty "Molecular Biology".
The indicated scientific contributions hold significant fundamental and scientific-applied relevance for the development primarily of molecular and cellular biology, biochemistry, and medicine.
The presented scientific works and contributions demonstrate a thorough and precise study of biological processes with significant innovative importance for science. The development of valuable tools, such as unique software and an open-access database, further highlights the great significance of Senior Assistant Professor Dr. Radoslav Alexandrov's scientific work.

Considering the above, I will confidently vote positively in the scientific jury for awarding Dr. Radoslav Alexandrov the scientific title of "Associate Professor".

30.04.2025 Prof. Dr. Rumiana Tzoneva

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