REVIEW

By: Acad. Prof. Ilza Konstantinova Pajeva, D.Sci., Institute of Biophysics and Biomedical Engineering – Bulgarian Academy of Sciences, Member of the Scientific Jury according to Order № 23-ОБ/ 21. 01. 2025 of the Director of the Institute of Molecular Biology "Acad. Rumen Tsanev" (IMB) - BAS

Regarding: Competition for the academic position of "Associate Professor" in: Field of higher education 4. *Natural Sciences, Mathematics, and Informatics*; Professional direction 4.3. *Biological Sciences*; Scientific specialty *Molecular Biology* for the needs of the Laboratory of Genome Stability at IMB-BAS, announced in the State Gazette No. 104/10.12.2024.

The sole candidate participating in the competition is **Senior Assistant Professor Dr. Radoslav Alexandrov Alexandrov** from the Laboratory of Genome Stability at IMB-BAS, Sofia.

1. Education and Professional Development. Radoslav Alexandrov holds a Bachelor's degree in Molecular Biology (2012) and a Master's degree in Biochemistry (2014) from the Faculty of Biology at Sofia University "St. Kliment Ohridski". He did his Master thesis work at the University of Poitiers, France as part of a student scientific exchange under the Erasmus program. In 2014, he joined the Laboratory of Genome Stability at IMB, where in 2018 he received a Ph.D. on a dissertation entitled "*Dynamics and Sequence of Binding of Proteins Involved in DNA Repair*" under the supervision of Assoc. Prof. Dr. Stoyno Stoynov. Based on the results of the dissertation, he published two articles in prestigious scientific journals (*Nature Communications* and *Molecular Cell*), thus demonstrating his commitment to achieving high-value scientific results. His work on the subject of the dissertation continued in his research after the defense and constitutes a significant part of his scientific contributions for this competition. Since February 2022, Dr. Alexandrov has been a senior assistant at IMB, with over 10 years of work experience in Molecular Biology, more than 2 of which he has been a senior assistant professor. His educational and professional qualifications so far are in line with the scientific field of the competition and testifie purposefulness of his scientific interests.

2. Analysis of the Candidate's Documents and Assessment of Compliance with the Requirements. Senior Assistant Professor Dr. Radoslav Alexandrov has submitted all necessary papers for the competition according to Article 5 of the "Conditions and Procedure for Holding the Academic Position 'ASSOCIATE PROFESSOR'" of the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria (PPZRASRB) at IMB - BAS (for brevity, hereafter referred to as the "IMB-BAS Regulations"). Below, they are summarized in accordance with the indicators in Table 1, Appendix 1 of the IMB-BAS Regulations.

- Group A (Indicator 1). In this group, the PhD dissertation on the topic "Dynamics and Sequence of Binding of Proteins Involved in DNA Repair" gives 50 pnts. The scientific articles used for obtaining the PhD degree do not double those used in the competition.
- Group B (Indicator 4). Four scientific publications indexed in Scopus/Web of Science each of quartile Q1 give in total 100 pnts (equal to the minimum).
- Group Г (Indicator 7). Eleven scientific publications, excluding those in Group B, of which 10 indexed in Scopus/Web of Science as follows: seven in Q1; one in Q2; one in Q3; and one in a publication with SJR, result in 220 pnts (equal to the minimum).
- Group Д (Indicator 11). 530 citations from the Scopus database (as of January 8, 2025) give 1060 pnts (at a minimum of 60 points).
- Group E (Indicators 14, 15, 16, 17, and 18). Participant in 11 national scientific projects (110 points out of a minimum of 10 points); participant in one international project (20 points out of a minimum of 20 points); head of two national scientific projects (40 pnts out of a minimum of

20 pnts); head of one international scientific project (50 points out of a minimum of 50 pnts); attracted funds through projects (268 pnts); in total 488 pnts.

In summary, according to the above listed indicators, Dr. Alexandrov accumulates 1918 points, significantly exceeding the minimum requirements for the academic position of "Associate Professor" according to the IMB-BAS Regulations (min. 430 points).

3. Contributions from the Candidate's Research Activities. Dr. Alexandrov has systematically outlined his main scientific contributions in five main groups, detailed in the Extended habilitation report, as well as summarized in the Brief report on scientific contributions. Based on this systematization, I have divided the contributions into two main groups in the further analysis:

I. Contributions related to studies of protein dynamics in living cells: 6 contributions are outlined, distributed in two subgroups based on a total of 10 scientific publications, including 8 original research papers and 2 review articles.

II. Other contributions (outside those in I): 3 contributions are outlined based on a total of 5 original research articles.

I. Contributions related to studies of protein dynamics in living cells.

I.1. Investigation of DNA repair dynamics in living cells.

I.1.1. The role of PARP-1 and effects of PARP-1 inhibitors.

Using tools developed in the Laboratory of Genome Stability for studying dynamics of fluorescently labeled proteins in living cells an original approach has been developed that reveals the complex nature of the process by which PARP-1 exerts its effect on damaged DNA molecules and simultaneously allows for an objective quantitative assessment of the capacity of PARP-1 inhibitors (PARPis) to retain DNA repair. For this purpose, three experimentally measurable parameters were defined: (1) PARP1 Retention Coefficient, PRC; (2) PARP1 Trapping Coefficient, PTC); (3) PARP1 Inhibition Coefficient, PIC. The concentration dependence of these parameters was investigated for a group of PARPis, and correlations between these parameters were derived, classifying the inhibitors according to their effects observed in the clinic. In particular, the retention coefficient PRC can serve as a reliable indicator for preclinical and clinical evaluation of PARPi. The advantage of the proposed approach is that it accounts for the "live" integral effect of PARPis in the natural environment of chromatin, i.e. simultaneously the catalytic inhibition of the enzyme and the allosteric capture of DNA, both as key factors for the inhibitory activity of the compounds. In fact, that's the advantage of this approach over the structure-based ones, which, in turn, allows for assessment of differences in the structures of various PARPis in relation of their impact on the allosteric domain (HD, helical domain) of PARP-1. The proposed approach can be extrapolated to investigate other inhibitors used for repairing damaged DNA molecules.

Research on this contribution has been published in the following article:

- Kanev, P.B., Varhoshkova, S., Georgieva, I., Lukarska, M., Kirova, D., Danovski, G., Stoynov, S., and Aleksandrov, R., 2024. A unified mechanism for PARP inhibito-induced PARP1 chromatin retention at DNA damage sites in living cells. Cell Reports, 43(5).

Questions: 1. According to the results, all studied compounds are PARP-1 inhibitors, i.e., they lead to decreased turnover rate of PARP-1 at damaged DNA sites. According to the studies of Zandarashvili et al. (*Science*, 2020), some of them (niraparib and veliparib) also exhibit allosteric effects by inducing changes in the HD domain, but in the opposite direction, i.e., facilitating the release of PARP-1 from DNA. How would you explain this difference?

2. How would you explain the behavior of the outliers (rucaparib and niraparib) in the correlation between PRC and the occurrence of DNA repair events over time (Fig. 6B in the publication)?

I.1.2. Validation of a mechanism for repairing damaged DNA by PARP-1.

By studying the dynamics of PARP-1 wild type and mutants in living cells, the mechanism by which PARP-1 repairs DNA was validated (initially established through biochemical

approaches by researchers from the Technical University of Dresden). It involves the formation of a so-called PARP-1-DNA condensate, where initially, via a PARP-1 dimer, followed by a tetramer, and subsequently through participation of many enzyme molecules, an ensemble (multimer) of PARP-1 molecules is formed around the broken ends of the DNA molecule, thereby holding them together. The assembly of multiple PARP-1 molecules around the broken ends of DNA is followed by processes of releasing the DNA ends (synthesis of PAR chains due to the activation of PARP-1) and stabilization (attraction and binding of the PAR-dependent protein *Fused in Sarcoma* (FUS), to the condensate and accumulation of additional PAR-dependent proteins that support the effective ligation of the broken DNA ends). These results not only validate the mechanism by which PARP-1 contributes to the repair of damaged DNA, but also helps building a more complete understanding of this mechanism.

Research on this contribution has been published in the following article:

• Chappidi, N., Quail, T., Doll, S., Vogel, L.T., Aleksandrov, R., Felekyan, S., Kühnemuth, R., Stoynov, S., Seidel, C.A., Brugués, J., Jahnel, M., Franzmann, T., and Alberti, S., 2024. PARP1-DNA co-condensation drives DNA repair site assembly to prevent disjunction of broken DNA ends. Cell, 187(4), pp.945-961.

Question: Is the nature of the interactions that occur between the domains of PARP-1 during the formation of multimers of PARP-1 molecules known?

I.1.3. New mechanism for spreading of chromatin phosphorylation. The study relates to another way, different from PARP-1, for recognizing double-strand breaks in DNA, which is carried out by the heterotrimeric MRN complex. In addition to the mechanism of spreading through loop extrusion, a mechanism of spreading of chromatin phosphorylation has been proposed, determining the crucial role of diffusion of ATM kinase molecules activated by the MRN complex. ATM molecules are inactive in the absence of double-strand breaks in DNA, but upon binding of the MRN complex to the damage sites, the latter attracts ATM kinases and activates them. Activated ATMs detach from the damage site and diffuses through the chromatin, phosphorylating histone H2AX (γH2AX) and attracting the regulatory protein MDC1 creating . To confirm this mechanism, a quantitative model was created that describes the dynamics of ATM propagation and the subsequent distribution of γH2AX/MDC1 to damaged DNA sites.

Research on this contribution has been published in the following article:

• Danovski, G., Panova, G., Keister, B., Georgiev, G., Atemin, A., Uzunova, S., Stamatov, R., Kanev, P.B., Aleksandrov, R., Blagoev, K.B., and Stoynov, S.S., 2024. Diffusion of activated ATM explains γH2AX and MDC1 spread beyond the DNA damage site. iScience, 27(9).

I.1.4. Development of specialized software tools for analyzing DNA repair dynamics.

The software CellTool and the database DNARepairK have methodological significance. Both tools have been developed in the Laboratory of Genome Stability at IMB-BAS. The software allows registration, segmentation, tracking of localizations, and extraction of results of DNA damage in living cells. In this way, a unique set of data obtained with high resolution is collected on the complex kinetic behavior of 70 proteins. The data were modeled using so-called CRC (Consecutive Reactions Chain) mathematical models. The models are implemented in the software and can be readily used. It is also possible to introduce entirely new models depending on the task of the specific analysis. For this purpose, the software platform MolDViewer is used, which allows users to upload their own data and apply the models of CellTool or other mathematical models to theoretically describe their data. Information about the effects of antitumor drugs on the dynamics of processes in DNA damage is also available. It is important to note that DNARepairK provides data from the study of the dynamic response of living cells to DNA damage, which cannot be achieved with other methods and make them unique. The software and database are freely accessible from the Laboratory's website at:

https://dnarepair.bas.bg/software/CellTool and http://dnarepair.bas.bg/index.php/dnarepairk/ and can be used by other laboratories abroad conducting such analyses.

Research on this contribution has been published in the following articles:

- Babukov, Y., Aleksandrov, R., Ivanova, A., Atemin, A., and Stoynov, S., 2021. DNArepairK: An interactive database for exploring the impact of anticancer drugs onto the dynamics of DNA repair proteins. Biomedicines, 9(9), p.1238.
- Danovski, G., Dyankova-Danovska, T., Stamatov, R., Aleksandrov, R., Kanev, P.-B., and Stoynov, S. (2023). CellTool: An Open-Source Software Combining Bio-Image Analysis and Mathematical Modeling for the Study of DNA Repair Dynamics. Int. J. Mol. Sci. 24, 16784. 10.3390/ijms242316784.

On the contributions in I.1, a review article has also been published, in which Dr. Alexandrov is the corresponding author:

- Kanev, P.B., Atemin, A., Stoynov, S., Aleksandrov, R., 2024. PARP1 roles in DNA repair and DNA replication: The basi (c) s of PARP inhibitor efficacy and resistance. Seminars in Oncology 51(1-2), 2-18.

Question: The data in DNArepairK were obtained with HeLa as the most widely used cell model for such studies. Do you plan to study the dynamics of proteins involved in DNA repair in other cell lines, and will the CRC models implemented in the software change when using other cells?

I.2. Investigation of the dynamics of DNA replication in living cells.

I.2.1. Investigation of the behavior of replication forks under replication stress.

At the single-cell level, through simulating replication stress (inhibition of the ATR kinase - a key enzyme from the DNA repair system) and tracking the behavior of fluorescently labeled proteins PCNA and RPA1, regulated by ATR, the dynamics of stalling and restart of replication forks in the dsame cellhave been revealed. Two processes were identified during fork stalling: one associated with suppression of DNA synthesis through rapid removal of PCNA, and another related to the gradual accumulation of RPA1 on nucleotides at the forks. Restart of forks under inhibited ATR leads to nucleotide retention, respectively to inability for DNA repair and cell death. In this way, the behavior of forks upon nucleotide depletion has been established. Investigation of the dynamics of proteins involved in regulating the behavior of replication forks is proposed as a tool for studying the effects of antitumor agents.

Research on this contribution has been published in the article:

- Dyankova-Danovska, T., Uzunova, S., Danovski, G., Stamatov, R., Kanev, P.B., Atemin, A., Ivanova, A., Aleksandrov, R. and Stoyno Stoynov, 2025. In and out of Replication Stress: PCNA/RPA1-Based Dynamics of Fork Stalling and Restart in the Same Cell. International Journal of Molecular Sciences, 26(2), p.667

I.2.2. *Investigation of the regulation of cellular morphology under replication stress conditions.*

By studying the effect of the genome stability protein Dia2 on the size of yeast cells, the mechanism by which this protein regulates cell length under replication stress conditions has been clarified. It has been shown that the absence of Dia2 increases the duration of the S- and G2/M-phases of the cell cycle through retaining the association between its substrate (the protein Ctf4) and chromatin. It has been established that Dia2 deficiency is the crucial condition for cell elongation and the duration of the cell cycle, while inhibiting DNA replication is the other necessary factor playing a role in the regulation of cellular morphology but with a significantly smaller effect compared to the absence of (or defects in) Dia2.

Research on this contribution has been published in the article:

- Ivanova, A., Atemin, A., Uzunova, S., Danovski, G., Aleksandrov, R., Stoynov, S. and Nedelcheva-Veleva, M., 2021. The effect of Dia2 protein deficiency on the cell cycle, cell size, and recruitment of Ctf4 protein in Saccharomyces cerevisiae. Molecules, 27(1), 97.

An overview article has also been published based on the contributions in section I.2.:

• Aleksandrov, R., Hristova, R., Stoynov, S. and Gospodinov, A., 2020. The chromatin response to double-strand DNA breaks and their repair. Cells, 9(8), p.1853

II. Other Contributions.

II.1. Investigation of the etiology of chronic rhinosinusitis.

The correlation between the formation of bacterial biofilms and the expression levels of two main types of mucins (MUC5AC and MUC5B) in the nasal mucosa, on the one hand, and the etiology of chronic rhinosinusitis, on the other, has been investigated in a group of 85 patients. For this purpose, confocal microscopy was used to determine the presence or absence of biofilms, and quantitative polymerase chain reaction (qRT-PCR) was employed to measure mucin expression levels. No significant correlation was found between colonization by bacterial biofilms on the upper respiratory tract mucosa and the gene expression levels of mucin glycoproteins. Significantly higher expression levels of MUC5B were found compared to MUC5AC. This contribution helps clarify the etiology of chronic rhinosinusitis and may have practical significance.

Research on this contribution has been published in the article:

• Popov, G., Aleksandrov, R., Petkova, V., Kaneva, R., Gergova, R., Kundurzhiev, T. and Popova, D., 2023. Analysis of bacterial biofilm formation and MUC5AC and MUC5B expression in chronic rhinosinusitis patients. Journal of Clinical Medicine, 12(5), p.1808

II.2. Investigation of metabolic differences between embryogenic and non-embryogenic plant cells.

Using a panel of cytological and biochemical methods, a new biological model comparing embryogenic and non-embryogenic plant cells from grapes with the same genetic origin has been proposed. It has been established that embryogenic plant cells are characterized by moderate cell proliferation, low intensity of glycolysis, and high oxygen consumption, determining the occurrence of active aerobic metabolism in these cells. In contrast, the metabolism of non-embryogenic cells is characterized by strong uncoordinated growth, intense glycolysis and fermentation processes, and lower oxygen consumption. These differences are attributed to variations in the utilization of resources from the nutrient medium and the organization and progression of cellular metabolism in both types of cells.

Research on this contribution has been published in the article:

• Parrilla, J., Gaillard, C., Verbeke, J., Maucourt, M., Aleksandrov, R.A., Thibault, F., Fleurat-Lessard, P., Gibon, Y., Rolin, D. and Atanassova, R., 2018. Comparative metabolomics and glycolysis enzyme profiling of embryogenic and nonembryogenic grape cells. FEBS Open Bio, 8(5), 784-798

II.3. Investigation of the mechanism and properties of the neurotoxin vipoxin, isolated from the snake Vipera ammodytes meridionalis.

Through comparative studies of the activity of unmodified and modified forms of the sPLA2 subunit (secretory phospholipase A2) of the heterodimeric protein complex vipoxin, the roles of amino acids His, Trp, and Lys for the catalytic activity and substrate binding of the enzyme were characterized. It has been found that the action of vipoxin results in destruction of cell membranes. Out of over 100 generated recombinant human antibodies scFv (single chain fragment variable), 33 recognize the toxin. Several of them have been identified to inhibit its hemolytic activity and could serve for developing effective antidotes.

Research on this contribution has been published in 3 articles:

• Danowski, G, Alexandrov, R, Pencheva, M, Petrova, S. CHEMICAL MODIFICATIONS OF PHOSPHOLIPASE A2 FROM VIPERA AMMODYTES: EFFECT ON CATALYTIC PROPERTIES. Science & Technologies, 3, 2013, 72-75

• Doumanov, J., Mladenova, K., Aleksandrov, R., Danovski, G. and Petrova, S., 2014. Interactions of pharmacologically active snake venom sPLA2 with different cell lines. Biotechnology & Biotechnological Equipment, 28(5), 918-922

• Stoyanova, V., Aleksandrov, R., Lukarska, M., Duhalov, D., Atanasov, V. and Petrova, S., 2012. Recognition of Vipera ammodytes meridionalis neurotoxin vipoxin and its components using phage-displayed scFv and polyclonal antivenom sera. Toxicon, 60(5), 802-809

Question: Do you plan to continue your research on vipoxin, and if so, in what direction?

4. Other activities of the candidate. These include: (a) participation in 14 national and international scientific forums with 8 oral communications and 6 posters; two of the oral communications were presented at prestigious scientific conferences in the USA and Bulgaria. (b) leadership of 2 national and one international project funded by the Swiss National Science Foundation (SNSF), starting in December 2024 for a 5-year period with a budget of 1,300,000 BGN; participation in 11 national and 1 international scientific projects; (c) Supervising 4 graduates - three bachelor's students from the Faculty of Biology at Sofia University and one master's student from Maastricht University, the Netherlands. In addition to the above described contributions, these appearances complete the scientific profile of the candidate and illustrate his multifaceted scientific and mentoring activities.

5. Evaluation of the quality and personal contribution of the candidate. I highly appreciate the scientific contributions of Dr. Alexandrov, especially those related to studying protein dynamics in reactions to damaged DNA in living cells. If it wasn't required by the PPRAASRB, I would accept only the publications and participations in scientific forums related to these contributions as sufficient material to evaluate his capacity for having the academic position of "Associate Professor". On this topic, he started working as a doctoral student in the Laboratory of Genome Stability at IMB-BAS, and this activity continues nowadays, which demonstrates his sustainable development as a researcher in this field. The high level of journals where Dr. Alexandrov and his colleagues, led by Assoc. Prof. Stoyno Stoynov, publish their results is noteworthy. In the competition, the candidate has presented a total of 15 scientific publications, out of which 13 are in journals indexed in Scopus/Web of Science. Their cumulative impact factor (IF) is 140.065, with one journal (Cell) having an IF of 45.5 (2023). The publications span the period from 2012 to 2025, with 9 of them being from the last 5 years (2020-2025). In two publications, he is either the first author or co-first author, and in two others, he is the corresponding author. Here, it is important to note that the candidate's position in the list of authors does not diminish his contribution, even if it is not the first or corresponding author. The nature of the research conducted in the Laboratory of Genome Stability (and not only there) suggests teamwork, where each participant contributes significantly to the overall results according to his/her specific expertise. The works in which Dr. Alexandrov is a co-author have been cited over 530 times (according to data from Scopus as of January 8, 2025), which, considering the short time frame of his publications, is a very good testament to the importance of their results.

For his achievements as a researcher, Dr. Alexandrov has been awarded numerous times, including the "Marin Drinov" Award for Young Scientists of BAS and the Award for the best scientific publication by a young scientist in the competition marking the 150th anniversary of BAS. In December last year Dr. Alexandrov won a project under his leadership from the SNSF through the program Promotion of Young Scientists in Central and Eastern Europe (PROMYS) titled "Deciphering DNA Damage Response Dynamics in Living Cells". This fact is an additional evidence of recognition of Dr. R. Alexandrov as an established scientist in this field. The project covers the main directions of his future research, outlined at the end of his Extended Habilitation Report.

6. Conclusion.

Senior Assistant Professor Dr. Radoslav Alexandrov Alexandrov meets the requirements for holding the academic position of "Associate Professor" according to Article 5 of the "Conditions

and Procedures for Holding the Academic Position 'ASSOCIATE PROFESSOR'" of PPZRASRB in IMB-BAS, significantly exceeding the minimum points required for this academic position. His results have been published in reputable scientific journals with high impact factors and have been cited multiple times, which testifies to their international recognition. He has original scientific contributions of fundamental character, as well as methodological ones and contributions with potential practical applicability. Dr. Alexandrov can be defined as a distinguished researcher with an established scientific profile and a clear vision for future development. His selection as an Associate Professor would contribute to the further professional development of the team at the Laboratory of Genome Stability at IMB-BAS. Based on the above, I strongly recommend to the esteemed Scientific Jury to vote positively and prepare a proposal to the Scientific Assembly of IMB-BAS for his appointment to the academic position of "Associate Professor" in the Field of higher education 4. *Natural Sciences, Mathematics, and Informatics*; Professional direction 4.3. *Biological Sciences*; Scientific specialty *Molecular Biology*.

25.4.2025

.../...

Ilza Pajeva