OPINION

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

Regarding the PhD Thesis of Georgi Yordanov Miloshev for the acquisition of the scientific and educational degree "doctor" in direction 4.3. Biological Sciences (Genetics-Bioinformatics) in the Department of Genetics at the Department of Genetics of the Faculty of Biology of the Sofia University "St. Kliment Ohridski" with scientific supervisor Prof. Dr. Robert Penchovski

Data on the doctoral student and the doctoral program.

Georgi Yordanov Miloshev graduated with a bachelor's degree in Molecular Biology at the Faculty of Biology of the Sofia University in 2017 and a master's degree in Genetics and Genomics at the same faculty in 2019. He is enrolled as a full-time doctoral student at the Department of Genetics of the Faculty of Biology, with order No. RD- 20-1188 / 08.07.2019, with the topic of the dissertation "Design and application of functional nucleic acids for synthetic control of gene expression" with scientific supervisor Prof. Dr. Robert Penchovski and dismissed by order No.RD-20-1188/11.07.2022. During the doctoral studies, the necessary number of exams have been passed and the necessary credits have been acquired according to the individual plan of the doctoral student. All deadlines and criteria according to the Regulations for the terms and conditions for acquiring scientific degrees and holding academic positions in the Sofia University, as well as the minimum national requirements according to the ZRASRB, have been met.

Dissertation Data:

Topic. The thesis topic "Design and application of functional nucleic acids for synthetic control of gene expression" reflects its content. A LacZ reporter gene in a plasmid expressed in Escherichia coli was chosen as a model for gene expression, and expression silencing was performed with a synthetic antisense oligonucleotide designed by the dissertation student and his supervisor.

Main parts of the dissertation.

The literature review examines in detail the importance of allosteric ribozymes and RNA-based technologies in modern synthetic biology, as well as their potential to create a new class of drugs that can directly regulate gene expression in target cells and thereby normalize their metabolism. A separate chapter is devoted to the use of RNA microarrays in the diagnosis and individual therapy of cancer diseases. This part is too general and with few specific references, making it somewhat vague and unfocused. Given the aim of the thesis, namely the control of gene expression in a prokaryotic model, it would be more appropriate for the review to focus on the use of allosteric ribozymes as a new class of antibacterial and antiviral agents rather than on various human cancers. The idea of directly influencing gene expression at the level of translation through riboswitches as well as at the level of transcription through interfering RNAs and microRNAs is not new, but is still far from clinical application due to the large volume of data to be analyzed and the multitude of factors , which affect each specific disease. Here I want to draw attention to the doctoral student to distinguish between "clinical application" and "clinical pathway" - terms that refer to different aspects of clinical practice (medical and financial). Any development that shows a direct application of this technique in experimental practice would be helpful in elucidating the mechanisms of action and applicability of the model.

The structure of hammerhead ribozymes and the main features of their design, as well as the potential mechanisms of their action both in the presence of RNAs indicative of a specific disease, and in the absence of RNA molecules important for normal cellular metabolism, have been examined in detail. A special chapter is devoted to the design and design of allosteric ribozymes, as well as to the types of allosteric ribozymes and methods of their selection. Attention is also paid to the control of gene expression based on ribozymes and antisense oligonucleotides. This part of the dissertation is spread over 62 pages and is illustrated with 22 figures, the sources of which are properly cited.

The aim of the doctoral thesis is to create a new universal method for controlling gene expression in the Escherichia coli model system by using synthetic antisense oligonucleotides that inhibit LacZ expression. For the fulfillment of this goal, 6 tasks have been formulated, which include both bio-informatics and molecular-biological methods at a modern level. Multiple databases and programs were used to design the oligonucleotides themselves and the gene silencing strategy. Laboratory equipment is described in excessive detail, including conventional equipment such as autoclave, refrigerator, freezer, balance, vortex, etc., with a

description of what they are used for. I recommend that in the next scientific text the mention of a laboratory technique be limited to a unique one. The methods and protocols used are presented as working protocols, often in milliliters rather than final concentration, as is accepted and more convenient for reproducing the experiments when necessary. The methods described are adequate to the tasks at hand and can be reproduced.

The results are accompanied by a theoretical justification of the model system used, which could also be considered as a preliminary discussion in view of studies published by other authors based on LacZ gene expression in Escherichia coli. The creation and restriction mapping of the plasmid is described as a first result, but incorrect expressions are allowed (for example, instead of "endonuclease site" in the description of Figure 24 and 26, it is mentioned "KpnI endonuclease", which is not correct). In this part, the individual steps of the protocol are again described, which should be in the Materials and methods chapter, and only the specific results should be commented on in the Results chapter. The resulting constructs were transfected into bacteria and propagated, and clones were then selected and confirmed by sequencing. Silencing of gene expression was done by antisense oligonucleotides according to an original design and an original author's strategy that was tested experimentally. In this part, the PhD student has detailed the results of different concentrations of ASO on expression. It is noteworthy that this part of the work is described in the first person, singular, and usually biological developments are the result of collective work. Given that all included dissertation publications are by more than one author, I recommend that in future scholarly writings the PhD student adheres to the more correct plural. This section also lacks a discussion of results as compared with similar or related scientific publications. At the end of the dissertation, a general discussion is made separately, which lacks any citations and has the character of a conclusion and summary.

Five conclusions were formulated that summarize the achieved results of the individual tasks and two contributions of a theoretical-applied nature. At the end is a list of abbreviations, as well as lists of tables, the latter of which I consider superfluous.

Scientific apparatus. 184 sources are cited, including the articles with the participation of the doctoral student in connection with the dissertation, which is unusual. It is agreed that these papers should be given in a separate list (which is also done) and not cited in the text, since they include the presented new results. The remaining 181 sources are contemporary and adequate to the topic of the dissertation.

Abstract. The abstract contains 71 pages and essentially reflects the content of the dissertation work. The parts included are Introduction, Aim and objectives, Materials and methods, Results, Discussion, Conclusions and Contributions. The mentioned technical notes to the dissertation also apply to the abstract.

Publications. The doctoral student has submitted a list of a total of three publications on the topic of the dissertation - two articles in periodicals and one chapter of an e-book. The two articles are in Q3 and Q4 quartile journals, which carry a total of 27 points, and the book chapter another 15 points, making a total of 42 points and thus exceeding the minimum number of 30 points for strand 4.3. Biological sciences, according to Appendix 1 of the ZRASRB. In one of the articles, the doctoral student is the first author, and in the other - the second, which is an indicator of the substantial contribution in the experimental data and obtained results included in the respective publications.

Conclusion. The thesis submitted for opinion is devoted to a rapidly developing area of biomedical research, namely the search for new antimicrobials. The design and experimental setups included in the dissertation are innovative and provide hope for the development of efficient strategies to combat multidrug resistance, which is a rapidly growing threat to human health. Despite the technical remarks made regarding the layout of the text, the work is valuable and contributes to the development of biomedical science. The requirements of ZRASRB for awarding the scientific and educational degree "doctor" have been met, and I give my positive assessment regarding the awarding of the scientific and educational degree "doctor" to Georgi Yordanov Miloshev.

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