

Szczecin, 15th April, 2022

**The review of the doctoral thesis of Mr. Krzysztof Kubiak, M.Sc. entitled “Mechanism of ColE1 plasmid replication by the Hfq protein of *Escherichia coli*” under the supervision of Prof. dr hab. Grzegorz Węgrzyn from the Department of Molecular Biology of the University of Gdańsk (and the co-supervisor Prof. Veronique Arluison). The review has been prepared due to the vocation of the Biology Discipline Council of the University of Gdańsk dated at 7<sup>th</sup> April 2022, signed by dr hab. Joanna N. Izdebska, prof. UG.**

## **I. Basic characteristic of the doctoral thesis**

The doctoral thesis of Mr. Krzysztof Kubiak, M.Sc. is composed out of five papers published in prestigious journals in the field of biology. Out of 5 papers, 1 is a review, 1 is a methodology paper and 3 are original manuscripts. It is important to note, that – as claimed – the authorship and contribution of Mr. Krzysztof Kubiak, M.Sc. was pivotal for the creation of these papers.


Here is the list of the papers, with Mr. Krzysztof Kubiak, M.Sc. contribution:

1. Cech G.M., Szalewska-Palasz A., **Kubiak K.**, Malabirade A., Grange W., Arluison V., Węgrzyn G. (2016) *The Escherichia Coli Hfq Protein: An Unattended DNA-Transactions Regulator. Front. Mol. Biosci. 3:36*

This is a review paper, introducing the subject of the doctoral dissertation. Mr. Krzysztof Kubiak, M.Sc. is a third Author in this paper, and his contribution was general assembly of the text of the manuscript.

2. **Kubiak K.**, Gaffke L., Pierzynowska K., Cyske Z., Grabowski Ł., Kosznik-Kwaśnicka K., Jaroszewicz W., Węgrzyn A., Węgrzyn G. (2022) *Determination of effects and mechanisms of action of bacterial amyloids on antibiotic resistance. Methods in Molecular Biology (in press)*

This is a methodological article describing the techniques used to test the involvement of bacterial amyloid proteins in the antibiotic resistance processes. For the moment of




the review, the paper is accepted for publishing in *Methods on Molecular Biology*, with the agreement attached to the documents. In this paper Mr. Krzysztof Kubiak, M.Sc. is the first Author and – as declared – participated in the preparation of the materials and methods section for chapters 2.3 and 3.3 (Estimation on mRNA levels and stability). Moreover, he participated in preparation of Quantification of level of antibiotic resistance in solid medium section for Chapter 3.2. (Determination of antibiotic resistance of bacteria).

3. Malabirade A., Jiang K., **Kubiak K.**, Diaz-Mendoza A., Liu F., van Kan J.A., Berret J.-F., Arluison V., and van der Maarel J.R.C. (2017) *Compaction and condensation of DNA mediated by the C-terminal domain of Hfq*. *Nucleic Acids Res.* 45: 7299-7308

This is an original paper that started the research on possible interaction of the Hfq protein with DNA and the effects of such interaction on the structure of nucleic acid. This paper was performed in the international team with the highly valued co-supervisor of Mr. Kubiak, MSc. PhD thesis – Prof. Véronique Arluison. Mr. Krzysztof Kubiak, M.Sc. is the third author in this paper and claimed to prepare and perform EMSA experiment. He also participated in the analysis and interpretation and visualization of EMSA data and prepared the first version of the manuscript.

4. Gaffke L., **Kubiak K.**, Cyske Z. and Wegrzyn G. (2021) *Differential Chromosome- and Plasmid-Borne Resistance of Escherichia coli Hfq Mutants to High Concentrations of Various Antibiotics*. *Int. J. Mol. Sci.* 22: 8886

This is an experimental paper on the role of Hfq protein and its C-terminal domain in the regulation of bacterial resistance to antibiotics. In this paper Mr. Kubiak, M.Sc. is the second author, with the equal contribution as the first author. His participation on the paper is significant – co-planning the experiments; constructing chromosomal mutants of *E.coli* within the Hfq protein; carrying out the primary experiments of chromosomal and plasmid born resistance and the qPCR and Western blott; performing transformation efficiency experiment for pSC101, pBR322 and pBR322 $\Delta$ rom plasmids; participating in the analysis and interpretation of the data, preparation of the initial version of the manuscript and visualization of the results.

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5. Wien F., **Kubiak K.**, Turbant F., Mosca K., Węgrzyn G., Arluison V. (2022) *Synchrotron Radiation Circular Dichroism, a new tool to probe interactions between nucleic acids involved in the control of ColE1-type plasmid replication. Appl. Sci. 12: 2639*


This is the last experimental paper in the doctoral dissertation of Mr. Krzysztof Kubiak, M.Sc. describing interactions of C-terminal domain of the Hfq protein with RNA molecules and a DNA fragment involved in the replication regulation of the ColE1 plasmid, with the use of Synchrotron Radiation Circular Dichroism (SRCD).

In this paper Mr. Krzysztof Kubiak, M.Sc. is the second Author and his contribution to the publishing of this paper is also crucial, such as designing nucleic acids particles used in the experiment, creating figure 1 and 2 and participating in the preparation of the initial version of the manuscript.

The overall scientific soundness of the papers included in the doctoral dissertation of Mr. Krzysztof Kubiak, M.Sc. is very high. All papers are published (or accepted to be published) in highly recognized scientific journals from the discipline of biology, listed on JCR list. In my opinion the construction of the doctoral dissertation, including a review paper, methodology paper and original papers exhausting the topic of the dissertation is very proper. The subject of the doctoral dissertation is crucial and updated. Exploring the role of *E.coli* Hfq protein, as pivotal on the processes related to virulence of pathogenic bacteria, seems to be important in the search for new antibacterial drugs. In the XXI century the problem of bacteria's resistance to antibiotics is one of the biggest threats for the human population. Scientists do their best to improve the knowledge of the biological basis of the process and search for alternative way to cope with the antibacterial resistance. The results of Mr.'s Krzysztof Kubiak, M.Sc. doctoral dissertation may have a great impact on the direction of the search for those new substances.

It is worth noticing, that by including a review paper into the doctoral dissertation, Mr. Krzysztof Kubiak, MSc. gave a wonderful evidence of his understanding of the role and importance of *E.coli* Hfq protein. I also think it is valuable, that the review has been created as the first, in 2016, showing the pathway and the direction of the future studies, that turned out to be a doctoral thesis. Moreover, having a review paper published is a proof of a great knowledge of the literature of the studies subject.

An important advantage of the doctoral dissertation of Mr. Krzysztof Kubiak, M.Sc. is also the variety of methods used in the experimental papers introduced in the selection. From



the electrophoretic mobility shift assay (EMSA), through constructing mutants, transformation efficiency, to qPCR and Western blott and more (including SRCD).

The overall impression of the doctoral dissertation is very positive, all the stated above arguments confirm the high methodological and substantive preparation of Mr. Krzysztof Kubiak, MSc.

## **II. Description of the papers included into the doctoral dissertation**


It is worth mentioning, that the doctoral dissertation, no matter the fact, that it is prepared as a selection of the published (accepted to be published) papers, has been prepared with a large dose of diligence. The Author has included an abstract in Polish and English, which is a brilliant summary of the most important data included in the papers, together with all needed documents, certifying the participation of the Author in the process of creating those pieces of science.

**II.1. Paper 1:** *Cech G.M., Szalewska-Palasz A., Kubiak K., Malabirade A., Grange W., Arluison V., Węgrzyn G. (2016) The Escherichia Coli Hfq Protein: An Unattended DNA-Transactions Regulator. Front. Mol. Biosci. 3:36*

This review is a complex summary of the properties of Hfq protein, with a special attention to regulatory role of Hfq protein in bacterial gene expression. The paper discusses the direct and indirect interactions between Hfq and DNA, impacting the role in – among others – plasmid replication. In the conclusion of the paper, the Authors clearly stated that further studies are needed to explore the interactions, and this is what Mr. Krzysztof Kubiak, M.Sc. achieved in the doctoral dissertation.

**II.2. Paper 2:** *Kubiak K., Gaffke L., Pierzynowska K., Cyske Z., Grabowski Ł., Kosznik-Kwaśnicka K., Jaroszewicz W., Węgrzyn A., Węgrzyn G. (2022) Determination of effects and mechanisms of action of bacterial amyloids on antibiotic resistance. Methods in Molecular Biology (in press)*

This paper is a good evidence of Mr. Krzysztof Kubiak, M.Sc. involvement in the methodological part of the scientific creation of the papers. The paper discusses the methods that can be used to study effects and mechanisms of action of bacterial amyloids on antibiotic resistance. The methods are needed to detect the assessment of formation of biofilms,



determination of efficiency of antibiotic resistance in solid and liquid media, determination of the effect on the gene expression at levels of abundance and stability of mRNAs and proteins.


**II.3. Paper 3:** *Malabirade A., Jiang K., Kubiak K., Diaz-Mendoza A., Liu F., van Kam J.A., Berret J.-F., Arluison V., and van der Maarel J.R.C. (2017) Compaction and condensation of DNA mediated by the C-terminal domain of Hfq. Nucleic Acids Res. 45: 7299-7308*

This paper allowed the Authors (among them, Mr. Krzysztof Kubiak, M.Sc.) to draw conclusions on the model for binding and bridging of Hfq on the double helix. The propensity for bridging, and compaction of DNA by the C-terminal domain might be related to aggregation of bound protein and may have implications for protein binding related gene regulation. This is an important observation, having an impact on the mechanisms behind the regulatory role of Hfq. Therefore, a new perspective has been opened for an in-depth study of the use of Hfq, considering those properties, discovered by the Authors of this paper.

**II.4. Paper 4:** *Gaffke L., Kubiak K., Cyske Z. and Wegrzyn G. (2021) Differential Chromosome- and Plasmid-Borne Resistance of Escherichia coli Hfq Mutants to High Concentrations of Various Antibiotics. Int. J. Mol. Sci. 22: 8886*

In this paper, the Authors found that the presence of Hfq is required for survival of plasmid-containing *Escherichia coli* cells against high concentrations of chloramphenicol (plasmid p27cmr), tetracycline (pSC101, pBR322) and ampicillin (pBR322). Moreover, from this study a conclusion may be drawn, that Hfq may participate in the control of ColE1-like plasmid DNA replication, with the special attention to the initiation stage and during plasmid establishment after transformation of bacterial cells. This paper is a great contribution to the state of knowledge on the antimicrobial resistance issue, since the results with mutant devoid of the part of the *hfq* gene coding for C-terminal region of Hfq suggested that this region (together with the N-terminal region) may be involved in the regulation of expression of antibiotic resistance in *E.coli*.





**II.5. Paper 5:** *Wien F., Kubiak K., Turbant F., Mosca K., Węgrzyn G., Arluison V. (2022) Synchrotron Radiation Circular Dichroism, a new tool to probe interactions between nucleic acids involved in the control of ColE1-type plasmid replication. Appl. Sci. 12: 2639*

The last paper in the doctoral dissertation selection of papers by Mr. Krzysztof Kubiak, M.Sc. In this study biophysical method was used, which is synchrotron radiation circular dichroism (SRCD). The use of this novel approach is very original and valuable, since the biochemical features of E.coli Hfq are usual and the more innovative method is used, the more applicative character it has. The aim of this paper was to investigate the interactions between RNA and DNA molecules involved in the regulation of the replication. The results obtained in the study are of a great biological value, as they assume to understand the ColE1 plasmid DNA replication in more detail. Moreover, the use of SRCD is very ingenious and innovative, worth appreciating.

Mr Krzysztof Kubiak, M.Sc. concluded the following, from the papers being his doctoral dissertation:

1. The Hfq protein, in particular its C-terminal domain, changes the mechanical properties and topology of the DNA double helix.
2. The efficiency of transformation of hfq mutants with ColE1-type plasmids is reduced, and interactions between Hfq and Rom in the regulation of replication of these plasmids are possible.
3. By stimulating the interaction between RNA I and RNA II, the Hfq protein, and in particular its C-terminal domain, can act as a negative regulator of the initiation of replication of ColE1- like plasmids.
4. The ColE1-like plasmids may overreplicate in E. coli cells, when the Hfq protein is inactive, leading to reduced viability of the bacteria.

The conclusions are drawn correctly on a good level of generality, but at the same time with details, important in the Author's research. The studies of Mr. Krzysztof Kubiak, M.Sc. are of a biological importance. The results constitute an important step forward towards deepening the knowledge of the biological properties of Hfq protein, and – by this – on the replication regulation of *Escherichia coli*.



### III. Final remarks and conclusion

The doctoral dissertation of Mr. Krzysztof Kubiak, M.Sc. entitled “Mechanism of ColE1 plasmid replication by the Hfq protein of *Escherichia coli*” is an original and complete selection of published (and accepted to publish) papers in well-recognized journals. The scientific soundness of the papers included in the selection is high. The conclusions constitute an important new element of biological knowledge in the field that has a great chance of being put into practice.

Bearing in mind all the advantages of the doctoral dissertation presented to me for evaluation, in particular:

- accurate verification of the research problem and the specific type of gap that the research fills;
- well-established knowledge of the research;
- properly planned and performed research experiments presented in the papers, with a wide range of used methods;
- correct confrontation and argumentation, as well as interpretation of the obtained results and skillful discussion;
- the appropriate language, specific for the subject of the research, the coherence of the texts;
- the ability to correctly draw conclusions,

I confirm that the doctoral dissertation of Mr. Krzysztof Kubiak, MSc., written under the supervision of Prof. dr hab. Grzegorz Węgrzyn and the co-supervision Prof. Veronique Arluison fully complies with the conditions set out in the Act of March 14, 2003 on academic degrees and academic title as well as degrees and title in the field of art (Journal of Laws 2003, No. 65, item 595, as amended).

In connection with the above, I would like to ask the High Council for Biology Discipline the University of Gdańsk for approval of Mr. Krzysztof Kubiak, M. Sc. for further procedure to obtain a doctoral degree.

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