Załącznik nr 3b

Bożena Nejman-Faleńczyk, PhD

THE DESCRIPTION OF SCIENTIFIC ACHIEVEMENTS

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Gdańsk, 2019

1. Full Name:

Bożena Nejman-Faleńczyk

2. Diplomas and academic degrees - with the name, place and year of obtaining them and the title of the doctoral dissertation

2012 r. Gdańsk – PhD in Biological Sciences in the field of Microbiology obtained at the Faculty of Biology of the University of Gdańsk, on June 15, 2012. My doctoral dissertation is entitled "Control of replication of Shiga toxin-converting phages in the light of potential new detection methods and therapies of enterohemorrhagic *Escherichia coli* infections" and was prepared under the supervision of Prof. Grzegorz Węgrzyn.

2010 r. Poznań – diploma of postgraduate studies in Dietetics and Nutrition Planning, obtained at the Faculty of Food and Nutrition Sciences of the Poznań University of Life Sciences. My thesis is entitled: "Dangers of food contamination with *Escherichia coli* O157:H7" and was prepared under the supervision of Prof. Józef Korczak.

2006 r. Toruń – MSc in Biotechnology, obtained at the Faculty of Biology and Earth Sciences of the Nicolaus Copernicus University in Toruń. My MSc thesis is entitled "TGF-β receptors expression determines progressive fibrosis" and was prepared under supervision of Prof. Anna Goc from UMK in Toruń and made in the laboratory of Prof. Oliver Eickelberg at the Justus Liebig University in Giessen, Germany.

2005 r. Giessen (Germany) – certificate of passing the first year of the international MBML program (Molecular Biology and Medicine of the Lung), intended for PhD students of the Medical School at the Justus Liebig University in Giessen, Germany.

2004 r. Toruń – Bachelor's degree in Biotechnology, obtained at the Faculty of Biology and Earth Sciences of the Nicolaus Copernicus University in Toruń. My BA thesis is entitled: "Characteristics of the human *BRCA1* gene" and was prepared under the supervision of Prof. Anna Goc.

3. Information about previous employment in scientific institutions

From 2012 – up to now (with a six-month break related to maternity leave) I am an adjunct at the Department of Molecular Biology (Faculty of Biology, University of Gdańsk)

4. Scientific achievement according to Art. 16 sec. 2 of the Act from March 14, 2003 on academic degrees and academic title as well as degrees and title in the field of art (Dz. U. 2017 r. poz. 1789):

a) Title of scientific achievement

Analysis of the function of selected, conserved regions of Stx phage genomes in the context of development of new detection methods and ways to treat infections caused by Shiga-toxigenic *Escherichia coli* strains

b) The list of publications included in the scientific achievement:

1. BLOCH SK, FELCZYKOWSKA A, **NEJMAN-FALEŃCZYK B#. (2012)** *Escherichia coli* O104:H4 outbreak--have we learnt a lesson from it? *Acta Biochimica Polonica*; 59(4): 483-488 – review
(#) – corresponding author

(IF_{2012} 1,185; MNiSW points: 15)

2. BLOCH S, **NEJMAN-FALEŃCZYK B**, ŁOŚ JM, BARAŃSKA S, ŁEPEK K, FELCZYKOWSKA A, ŁOŚ M, WĘGRZYN G, WĘGRZYN A. **(2013)** Genes from the *exo-xis* region of λ and Shiga toxin-converting bacteriophages influence lysogenization and prophage induction. *Archives of Microbiology* 195(10-11): 693-703. DOI: 10.1007/S00203-013-0920-8 – original paper

(IF_{2013} 1,861; MNiSW points: 20)

3. BLOCH S, **NEJMAN-FALEŃCZYK B,** DYDECKA A, ŁOŚ JM**,** FELCZYKOWSKA A, WĘGRZYN A**,** WĘGRZYN G. **(2014)** Different expression patterns of genes from the *exo-xis* region of bacteriophage lambda and Shiga toxin-converting bacteriophage Φ24B following infection or prophage induction in *Escherichia coli. PLoS One* 9(10): e108233. DOI: 10.1371/JOURNAL.PONE.0108233 – original paper

(IF_{2014} 3,234; MNiSW points: 40)

4. **NEJMAN-FALEŃCZYK B#**, BLOCH S, JANUSZKIEWICZ A, WĘGRZYN A, WĘGRZYN G. **(2015)** A simple and rapid procedure for the detection of genes encoding Shiga toxins and other specific DNA sequences. *Toxins (Basel)*. 7(11): 4745-4757. DOI:10.3390/TOXINS7114745 – original paper,
(#) – corresponding author

(IF_{2015} 3,571; MNiSW points: 30)

The method presented in this work has obtained national patent protection (Patents No. B1 218839 and No. B1 220906 are described in Appendix 4).

5. BLOCH S*****, **NEJMAN-FALEŃCZYK B*,** TOPKA G, DYDECKA A, LICZNERSKA K, NARAJCZYK M, NECEL A, WĘGRZYN A, WĘGRZYN G. **(2015)** UV-sensitivity of Shiga toxin-converting bacteriophage virions Φ24B, 933W, P22, P27 and P32. *Toxins (Basel)*. 7(9): 3727-3739. DOI: 10.3390/TOXINS7093727 - original paper, (*) – equivalent to the first authorship

(IF_{2015} 3,571; MNiSW points: 30)

6. **NEJMAN-FALEŃCZYK B**, BLOCH S, LICZNERSKA K, FELCZYKOWSKA A, DYDECKA A, WEGRZYN A, WEGRZYN G. **(2015)** Small regulatory RNAs in lambdoid bacteriophages and phage-derived plasmids: not only antisense. *Plasmid* 78: 71-78. DOI:10.1016/J.PLASMID.2014.07.006 - review
(IF_{2015} 1,732; MNiSW points: 15)

7. **NEJMAN-FALEŃCZYK B***, BLOCH S*, LICZNERSKA K, DYDECKA A, FELCZYKOWSKA A, TOPKA G, WĘGRZYN A, WĘGRZYN G. **(2015)** A small, microRNA-size, ribonucleic acid regulating gene expression and development of Shiga toxin-converting bacteriophage Φ24B. *Scientific Reports* 5: 10080. DOI: 10.1038/SREP10080 – original paper
(*) – equivalent to the first authorship
(IF_{2015} 5,228; MNiSW points: 40)

8. LICZNERSKA K, **NEJMAN-FALEŃCZYK B**, BLOCH S, DYDECKA A, TOPKA G, GĄSIOR T, WĘGRZYN A, WĘGRZYN G. **(2016)** Oxidative stress in Shiga toxin production by enterohemorrhagic *Escherichia coli*. *Oxid Med Cell Longev*. 2016: 3578368. DOI:10.1155/2016/3578368 – review
(IF_{2016} 4,593; MNiSW points: 30)

9. BLOCH S, WĘGRZYN A, WĘGRZYN G, **NEJMAN-FALEŃCZYK B#**. **(2017)** Small and smaller-sRNAs and microRNAs in the regulation of toxin gene expression in prokaryotic cells: A mini-review. *Toxins (Basel)*. 9(6): 181. DOI:10.3390/TOXINS9060181 – review
(#) - corresponding author
(IF_{2017} 3,273; MNiSW points: 35)

10. BLOCH S*, **NEJMAN-FALEŃCZYK B***, PIERZYNOWSKA K, PIOTROWSKA E, WEGRZYN A, MARMINON CH, BOUAZIZ, Z, NEBOIS, P, JOSE, J, LE BORGNE, M, SASO, L, WEGRZYN G. (**2018)** Inhibition of Shiga toxin-converting bacteriophage development by novel antioxidant compounds. *J Enzyme Inhib Med Chem*. 33(1): 639-650. DOI:10.1080/14756366.2018.1444610 – original paper
(*) – equivalent to the first authorship
(IF_{2017} 3,638; MNiSW points: 25)

11. DYDECKA A, **NEJMAN-FALEŃCZYK B**, BLOCH S, TOPKA G, NECEL A, DONALDSON LW, WĘGRZYN G, WĘGRZYN A. **(2018)** Roles of *orf*60a and *orf*61 in development of bacteriophages λ and $Φ24_B$. *Viruses*. 10(10): 553. DOI: 10.3390/V10100553 – original paper
(IF_{2017} 3,761; MNiSW points: 30)

The scientific achievement is a series of eleven, thematically related, scientific articles published in 2012-2018, in journals with Impact Factor (IF) and included in the Journal Citation Reports (JCR). All publications come from the period after the defense of my doctoral thesis, which took place in June 2012. IF were obtained from the JCR according to the year of publication of the paper or the last one available. The total IF for publications included in the scientific achievement is **35.6 (IF_{SUM})**. The MNiSW points were obtained on the basis of Ministry of Science and Higher Education list of articles, part A, according to the year of publication or the last one available. The sum of MNiSW points obtained for the above-mentioned articles is **310 ($MNiSW_{SUM}$)**.

Among the eleven publications, four are review papers, while the other seven describe original research. I'm the corresponding author (#) in three of the presented articles. In five of them I am the first or equally the first author (*) and in the remaining ones I occupy the second position. Importantly, as the second author, I served as an auxiliary supervisor of the author (PhD student) from the first position. A description of my individual contribution to each of the 11 articles can be found in Appendix No. 4. In turn, co-authors' statements about their contribution to the listed above publications can be found in Appendix No. 5.

In the further part of the self-presentation, the articles included in this scientific achievement are cited in accordance with the above numbering **[paper No. 1-11]**. The list of supplementary publications cited in the section 4c can be found under the description. Only the most important reports were mentioned below. The remaining essential references, can be found in the discussed papers.

c) Description of the aim and results of the above-mentioned publiactions and their potential practical significance

The main topic discussed in this scientific achievement is related to Stx bacteriophages transferring genes coding for dangerous to humans Shiga toxins. These phages infect *Escherichia coli* bacteria and thus transform them into toxin-producing pathogens. Among them, the most common are enterohaemorrhagic *E. coli* strains (EHEC). Research carried out in the frame of this achievement, draw attention to selected sequences conserved in the Stx phage genomes that may be important in detection and treatment of the infections caused by Shiga-toxigenic bacteria.

The most well-known representative of EHEC is *E. coli* O157:H7 recognizable worldwide since 1982. This bacterium was first identified in the USA, during an investigation of the outbreak of many cases of food poisoning with severe hemorrhagic diarrhea, associated with consumption of contaminated hamburgers in one of the fast food restaurants (Riley *et al.* 1983). At that time, it was not clear that the main cause of noticed symptoms were the Shiga toxins, which genes (*stx*) are located in the genome of the occurring in bacteria Stx prophage (but not in the genome of the bacteria itself). Besides, it was also not known that the production of toxins depends on the decision of phage to switch from lysogenic development into a lytic cycle.

Since then, over 470 different serotypes of *E. coli* bacteria able to produce Shiga toxins have been identified. All of them were infected with Stx phages. Every year, these pathogenic bacteria cause several hundred thousand cases of food poisoning and there is a significant increase in the number of infections caused by other than O157: H7, *E. coli* serotypes. (Lee *et al.* 2016). Epidemiological data indicate that the most common cause of infection is the consumption of contaminated beef and sprouts. Common sources of infections are also other raw vegetables, unpasteurized juices or water contaminated with animal feces, becoming from domestic animals (mainly cattle) that are the asymptomatic carriers of EHEC bacteria (Krüger and Lucchesi, 2015). The prevalence of these sources generates many problems and tremendous

economic losses that accompany every outbreak. They are associated with difficulties in recognizing the primary source of infection and public fears of consumption of fruits, vegetables and meat (mainly beef). For example, financial losses of food producers resulting from the outbreak in Germany and other European countries, in 2011, that was caused by Shiga-toxigenic *E. coli* O104:H4 strain, were estimated at over 3 billion EUR (according to WHO). The main cause of these fears is the awareness that there is no effective treatment for infection with Shiga toxin-producing bacteria, and untreated infections can lead to the development of severe complications, such as hemolytic uremic syndrome (HUS) occurring with acute renal failure. Unfortunately, many antibiotics and known antibacterial agents cannot be used in the treatment of this type of infection. Many of them have been classified as prophage inducers which activate SOS response, provoke phage lytic development and increase production of Shiga toxins, and thus enhance severity of disease symptoms (Kimmitt *et al.* 2000). In addition, the 2011 outbreak highlighted the serious problems associated with the detection of such infections as we analyzed in **paper No. 1.** As we pointed out in this work, the greatest diagnostic difficulty during this epidemic was due to the fact that the cause of numerous and rapidly progressing food poisoning cases was a bacterium with atypical features (*E. coli* O104:H4). It turned out that this bacterium had a unique set of virulence factors characteristic for both enterohaemorrhagic as well as enteroaggregative (EAEC) *E. coli* strains. Similarly to other EHEC bacteria, *E. coli* O104:H4 released the Shiga toxin, however unlike them, was able to produce neither the important for adherence intimin nor enterohemolysin. In return, O104:H4 strain had genes coding for adherence fimbria typical for EAEC bacteria. Finally, it was established that this bacterium can represent a new pathotype and that several horizontal gene transfer events took place to create its genome. Ability to produce Shiga toxin, the *E. coli* O104:H4 bacteria acquired as a result of infection with Stx phage carrying its genes (*stx*). Importantly, the analysis of the phage genomic sequence showed its high similarity to the sequences of Stx phages infecting bacteria from the EHEC group. Essentially, this was the first case of Stx phage infection of O104:H4 serotype. As a result of this, the diagnostic methods based on serogrouping were ineffective during this outbreak. In addition, molecular methods routinely used in detection of bacteria from the EHEC group and based on the identification of the intimin gene (*eae*), have also failed. Taking into account that other rare bacterial strains can also be converted into Shiga toxins producers, in **paper No. 1**, we indicated the need to develop new molecular diagnostic methods based on detection of sequences located on the mobile genetic elements, in particular the sequences of phage genes coding for Shiga toxins (*stx*).

We have made this work too **[paper No. 4]**. We analyzed the sequences of *stx* genes encoding Shiga toxins 1 and 2 and within them we identified regions that revealed a high degree of conservation among the Stx phage genomes. We have used these fragments of *stx1* and *stx2* genes and designed two sets of primers and probes recognizing them. As a result of our work, we obtained a new method based on PCR technology that allows for amplification and thus detection of the above-mentioned DNA sequences. The effectiveness and specificity of this procedure were tested on DNA samples obtained from 19 different isolates of Shiga-

toxigenic *E. coli* bacteria, that we received thanks to cooperation with Aleksandra Januszkiewicz, PhD from the National Institute of Hygiene. The method proposed by us resembles classical PCR tests, however, in this case, the amplification of a target DNA sequence (of the Shiga toxin gene 1 or 2) by DNA polymerase occurs in the presence of a pair of primers and a double-labeled, sequence-specific DNA probe. The probe is a short, complementary to the target sequence, DNA oligonucleotide having a UV-excited fluorescent reporter at one end, and compatible with it, a fluorescence quenching molecule at the other end. In turn, primers are designed to the DNA fragment that includes the probe binding site. In this procedure, we use the 5 '→ 3' exonuclease activity of *Taq* DNA polymerase, thanks to which, the probe after binding to the target DNA fragment, is degraded by the polymerase extending a specific primer designed for this sequence. As a result of the degradation, the reporter is released from the inhibitory action of the quencher and after excitation at the appropriate wavelength, emits a fluorescence signal. The mechanism described above was already known (Bustin, 2000) and used previously in real time PCR technique. However, according to the concept presented in **paper No. 4**, we decided to use this scheme in a PCR reaction carried out in classic thermocyclers. In contrast to the previous examples, we used not important in the case of real-time PCR reaction, ability of some reporters (e.g. FAM) to be excited under UV light. Thanks to this feature, we offered a new and alternative to the standard agarose gel DNA electrophoresis, way to detect DNA products that are generated in such reaction. This detection manner is very simple and takes only a few minutes. It assumes detection of the specific fluorescent signal becoming from the released reporter, by a simple observation of the post-reaction tube under UV light. Importantly, this signal is visible under UV as a color of the post-PCR mixture that appears only if amplification of the target DNA occur **[Figure 1 - paper No. 4]**. We have shown that in comparison with the classic PCR tests (that are commonly proposed in combination with the standard agarose gel electrophoresis as detection procedure), the manner we propose is not only faster but also more specific. Besides, due to the lack of the need to transfer the post- PCR mixture from the tube, it is also less susceptible to post-reaction contamination. In turn, in relation to real-time PCR tests, this method does not require a complicated analysis of results and the acquisition of an expensive real-time PCR device. Here, detection of the signal is possible by using a thermocycler and a simple source of UV light e.g. transilluminator. The proposed method was appreciated by the national institution, and I received funds for its development in the frame of the LIDER program of the National Center for Research and Development (No. Lider/21/92/L-3/11/NCBR/2012). In addition, since 2015 it has been covered by national patent protection [patents: II.B.1-2 Appendix no. 4]. Currently, I am continuing research in this topic and working on new variants of these tests allowing for detection of other pathogens. I carry out this research as part of the UG project named "Incubator of Innovation +" that is funded by Ministry of Science and higher Education (No. MNISW/2017/DIR/68/II +).

During the studies related to the detection method, we have additionally observed that Stx bacteriophages are much more sensitive to UV light than closely related λ phage. In **paper No. 5**, we showed

that UV irradiation (at a dose of 50 J/m^2) negatively affected the stability of Stx phage virions and their ability to infect *E. coli* bacteria. Analysis with the use of electron microscopy technique has shown that the cause of reduced stability of phages is most probably the UV-induced damage of the capsids leading, at least in some of the analyzed cases, to the leakage of phage DNA. On this basis, it would seem that UV light is a good tool to fight against bacteriophages that carry the Shiga toxins genes. Curiously, confirming previous reports (Aersten *et al.* 2005), we observed that the UV irradiation applied in the above dose also stimulates the induction of Stx prophage and its further lytic development in *E. coli* bacteria associated with the production of Shiga toxins **[paper No. 2]**. However, bearing in mind that it is unlikely that UV light occurs in the lower part of the human gastrointestinal tract and induces Stx prophages during infection, we decided to continue the previously started consideration (Łoś *et al.* 2013) on the role of hydrogen peroxide in this process. In **paper No. 8**, we referred to reports indicating that the hydrogen peroxide produced by neutrophils can be a natural inducer of the lytic development of Stx phages in the pathogenic *E. coli* bacteria, in human intestine. We also reminded that the mechanism of this induction is based on the degradation of the main repressor of phage lytic cycle - CI protein. Importantly, the CI degradation occurs as a result of the SOS response, which in turn is triggered by the appearance of single-stranded DNA fragments in the cell. These, ssDNA fragments, are result of the DNA damage that is induced in the presence of hydrogen peroxide, but also other factors, for example UV light. On the basis of available in the literature data and the observed in **paper No. 3** different expression patterns of phage genes following prophage induction with various agents, in the **paper No. 8**, we postulated that the efficiency of induction of lambdoid prophages, including Stx, caused by hydrogen peroxide is lower than this stimulated by antibiotics or UV light. We referred to data indicating that H_2O_2 caused initiation of the lytic phage development in about 1 % of cells in bacterial population, whereas mitomycin C induced prophages in around 30 % of the cells. Considering the mechanism responsible for the low efficiency of prophage induction under conditions of oxidative stress, we came across an interesting report (Glinkowska *et al.* 2010) indicating an important role of the OxyR protein which is activated under such conditions. This protein has been shown to bind in the promoter region of p_M-p_R λ phage and contributes to more efficient transcription from the p_M promoter and thus increased expression of the *cI* gene. Importantly, the *in silico* analyzes carried out by us as well as the authors of that report, showed the existence of potential binding sites for the OxyR protein within the p_M-p_R promoter regions of Stx phages. On this basis, in **paper No. 8,** we proposed a common for λ and Stx phages, OxyR-mediated mechanism of prophage induction with hydrogen peroxide. This mechanism assumes that under conditions of oxidative stress (caused e.g. by the presence of H_2O_2), the active OxyR protein contributes to the increased production of CI protein. Due to the large amount of CI, this protein is not completely degraded during the SOS response. As a result, in most cells of the bacterial population, this protein still functions and leads to a more efficient maintenance of prophage. In effect, the activation of the phage lytic cycle and the production of phage encoded proteins (including Shiga toxins) occurs only in a small part of the population. As a consequence of the production of Shiga toxins and new phage progeny release, the small

fraction of bacterial cells is destroyed. Curiously, the elimination of some bacterial cells is beneficial for the whole population (Łoś *et al.* 2013). As indicated previously, production of Shiga toxins is a kind of defense bacterial strategy against predators such as protozoan *Tetrahymena thermophila* and can lead to its death. In fact, *Tetrahymena* produces H_2O_2 to damage bacteria during attack and thus induces the defense strategy by itself (Lainhart *et al.* 2009). Importantly, due to the low effective prophage induction by H_2O_2, only 1 % of cells dies so the others can survive the attack of the predator. Interestingly, similar situation may occur in human intestine where neutrophils are also able to produce the hydrogen peroxide. In this light, the intestinal oxidative stress conditions appear to be the very likely signal for the production of Shiga toxins by pathogenic *E. coli* bacteria found in the human gastrointestinal tract. Due to this, it was crucial for us to investigate other regions of the Stx phage genome that may play important role in the phage response to the such conditions.

A fragment of the phage genomic sequence located between the *exo* and *xis* genes turned out to be such a region. As shown in **paper No. 2**, characteristic for the *exo-xis* region of λ and Stx phages, is occurrence of four open reading frames of unknown function (called in λ: *orf60a, orf63, orf61* and *orf73)* and one recognized but uncharacterized gene (*ea22*). Interestingly, in contrast to λ *exo-xis* region, the Stx phages contain additional open reading frames and do not have the equivalent of the *ea8.5* gene. Considering the evolutionary tendency to preserve the biologically significant sequences unchanged and on the basis of previous reports related to λ *exo-xis* (Łoś *et al.* 2008), we decided to investigate the impact of this region on the development of Stx phage (Φ24B) in *E. coli* bacteria. Initially, we conducted studies under the conditions of overexpression of the entire *exo-xis* region. We obtained such conditions by introducing into the bacterial cells a plasmid containing an additional copy of the fragment located between the *exo* and *xis* genes of Φ24B genome. As a result of this work, in the overexpression variant, we observed an increase in the number of phage particles released after prophage induction and further lytic development caused by both analyzed agents: mitomycin C and hydrogen peroxide. Interestingly, this effect was more evident in the experiment conducted at 30°C **[paper No. 3]** than 37°C **[paper No. 2]**. Moreover, we shown that after prophage induction, an increase in phage DNA amount was significantly higher in bacteria carrying an additional copy of the *exo-xis* region on the plasmid. Importantly, the survival rate of these bacteria was lower compared to the control **[paper No. 3].** As expected, the efficiency of lysogenization of *E. coli* bacteria by Φ24B and survival of bacteria after this infection were lower in the variant of *exo-xis* overexpression **[paper No. 2]**. In the light of the obtained results, we concluded that there is an important relation between the *exo-xis* region and the phage's lytic development. These observations were consistent with previous report that has shown that the presence of the λ *exo-xis* region on multicopy plasmid in the cell, resulted in the inhibition of transcription from promoters dependent on CII protein, such as p_E, p_I and p_{aQ}, which activity is important during the lysogenic cycle (Łoś *et al.* 2008). On this basis, we have decided to continue research in this topic. We prepared a scientific project that received funding in the Opus 5 competition of the National Science

Center (No. UMO-2013/09/B/NZ2/02366). As a co-author of the project, I took the position of the main investigator. In the frame of this project, we performed research using phage deletion mutants, devoid of sequences of the whole or a specific fragment of the *exo-xis* region. Such mutants were obtained by us by homologous recombination. As a result of our work, we've published the article [paper No. II.A.12, Appendix 4], which is not included in this achievement, however is thematically related to it and important from the point of view of this discussion. In this work we showed that the induction of phage Φ24BΔ*exo-xis*, devoid of the *exo-xis* region, by hydrogen peroxide, was drastically impaired. Interestingly, the detected in this variant amount of phage particles, obtained after prophage induction, was equal to this observed during spontaneous induction in control bacteria, cultured without an induction agent. Moreover, comparing to wild type phage, we noticed decreased expression of key phage genes after *E. coli* Φ24BΔ*exo-xis* induction with hydrogen peroxide. To our surprise, the level of expression of bacterial genes essential for the SOS response was also reduced in the deletion variant after treatment with hydrogen peroxide (but not UV light), suggesting that genes located in the *exo-xis* region are necessary during H_2O_2-mediated SOS response. Importantly, the described effects were less pronounced or even not observed in λ phage. Knowing this, we decided to take a closer look at the open reading frames located in the *exo-xis* region of phage Φ24B. Our attention was drawn to two sequences *vb_24B_9c* and *vb_24B_7c*. As they are highly similar to open reading frames identified in the *exo-xis* region of λ phage (*orf60a* and *orf61,* respectively), in our further work, we adopted names for them according to the nomenclature used in phage λ. In **paper No. 11**, we showed that both, the nucleotide and the predicted amino acid sequences of *orf60a* and *orf61* are highly conserved among Stx phages. In turn, when analyzing the development of phage deletion mutants lacking these sequences (Φ24BΔ*orf60a* and Φ24BΔ*orf61*) in *E. coli* bacteria, we noticed that both analyzed open reading frames are important during the induction of Φ24B prophage with hydrogen peroxide, as their absence negatively affected this process. Interestingly, the observed effect was not as spectacular as in the case of deletion of the entire *exo-xis* region from the Φ24B phage genome [paper No. II.A.12 Appendix 4]. In addition, despite of high sequence similarity to analogous open reading frames in the *exo-xis* region of λ phage, we did not observe such significant effects in the parallel experiment with the λΔ*orf60a* and λΔ*orf61* deletion mutants. We noticed also, that the increase in the efficiency of lysogenization of *E. coli* infected by phage deletion mutants, was more significant in the case of Φ24B phage than λ mutants. In the light of research carried out under conditions of either overexpression or deletion of the *exo-xis* region, we have suggested that this region plays an important role in the induction of Stx phages under oxidative stress conditions. The importance of the *orf60a* and *orf61* is significant in this process, although the impact of the entire *exo-xis* region is stronger and it is undoubtedly the result of the interaction of other factors encoded in this region, such as the also tested by us *orf63* [paper No. II.A.14, Appendix 4]. Importantly, this work is not included in the discussed achievement and is described in the next section. I mention it here, due to the fact that it is the first paper prepared in cooperation with Prof. Logan Donaldson from the University of York in Canada, with whom we investigated also the discussed above *orf60a* and *orf61*. Knowledge gained so far in this topic,

allowed us to realize that the understanding of the *exo-xis*-dependent mechanism of regulation of Stx phages development in *E. coli* bacteria under oxidative stress conditions, can have both cognitive and potential practical significance. Knowing that, the pathogenicity of Shiga-toxigenic bacteria in the intestine depends on the choice made by the bacteriophage at the stage of "lysis vs lysogenization" decisions, but also on the efficiency of prophage induction and its further lytic development under such conditions, we suggested that the obtained by us knowledge may be important in developing alternative treatment therapies.

We've also done an extra job in the context of research relevant to the development of new treatment strategies for this type of bacterial infection. Bearing in mind that, the state of oxidative stress is the result of the action of free radicals (including the highly reactive hydroxyl radical generated from hydrogen peroxide), which cause numerous damage to the cell, including DNA damage that induces the SOS response, in the next stage of our research **[paper No. 10]** we checked whether compounds with antioxidant properties can counteract the induction of Stx prophage under such conditions. Thanks to cooperation with the research group, of Prof. Luciano Saso from the Sapienza University in Rome, we acquired 46 newly synthesized compounds and analyzed their impact on the growth of *E. coli* bacteria lysogenic with Stx phage (Φ24B) after induction of the prophage. Interestingly, in comparison with control culture (DMSO), we observed a significant increase of bacterial growth in cultures carried out in the presence of the 15 different compounds, what suggested the possible inhibition of the induction and/or further lytic development of the Stx phage. In order to confirm our assumptions, we decided to focus on the 15 selected compounds and measure the titers of bacteriophages in cultures treated with different inducing agents (one of the tested was hydrogen peroxide). As a result of the analysis, we observed that despite the use of hydrogen peroxide, the number of phage particles was drastically reduced in the presence of all 15 analyzed compounds. Moreover, the analysis of gene expression in these conditions, carried out for three selected compounds, showed a significant decrease in the level of expression of oxidative stress genes, but also key phage lytic genes. In turn, the expression of the *cI* gene coding for the main repressor of the phage lytic development increased significantly. This proves the directed action of the tested compounds on phage development, and allow us to suppose that by reducing the oxidative stress in the cell, the analyzed compounds prevent the H_2O_2-depndent induction of Stx prophages. In effect, the lysogenic cycle is preferred and Shiga toxins are not produced. In practice, this creates the possibility of using these compounds as potential drugs against infections caused by Shiga-toxigenic bacteria.

Searching for other regions in Stx phage genomes that may be important in the developing of alternative treatment strategies for these infections, we took a closer look at the small regulatory RNA molecules of phage origin. Our choice was not accidental. Numerous examples of therapeutic significance of such molecules have been described in the literature so far (DeJong *et al.* 2002). In **paper No. 6,** we thoroughly analyzed two non-coding RNA molecules identified in phage λ genome (aQ RNA and oop RNA). We performed *in silico* analyzes and indicated on high similarities between sequences coding for oop RNAs

of λ and Stx phages. Besides, we proposed models of secondary structure for the identified oop RNAs. In addition, we have collected information about other, newly discovered in the genomes of Stx phages, small non-coding RNA molecules, which, as our search has shown, is quite a lot. Encouraged by these results, we also decided to look for new such molecules. As a result of our investigation **[paper No. 7]**, we discovered the first phage microRNA-size molecule, which we named 24B_1, in relation to the name of the bacteriophage Φ24B, in which we found it. The identified molecule has only 20 nucleotides and probably comes from a longer 80-nt long precursor transcript. The sequence coding for 24B_1 is located in the Φ24B phage genome between gene *lom* and the open reading frame *vb_24B_43*. Importantly, this sequence is highly conserved in the Stx phage genomes, however is absent in the λ phage genome. In addition to this, we identified two potential binding sites for this molecule in Φ24B genome. One of them is located upstream of the *S* gene, and the second within the *d_ant* gene, suggested to encode a protein of anti-repressor function. On this basis, we made a series of experiments using a phage deletion mutant lacking the sequence coding for this molecule, and showed its biological significance. We observed that phage devoid of 24B_1 coding sequence revealed decreased efficiency of lysogenization, quicker prophage induction, more efficient production of phage progeny during lytic cycle and less effective adsorption on the bacterial cell. Furthermore, in comparison with wild type phage, we noted a significantly increased level of expression of key phage genes during infection of bacterial cells with the Φ24BΔ24B_1 mutant. Considering the obtained results, we proposed the mechanism of 24B_1 action. This mechanism indicates the role of the 24B_1 molecule as a negative regulator of expression of *d_ant* gene which product in turn, may inactivate the main repressor of the phage lytic cycle, CI. As a consequence of its action, the anti-repressor protein is not produced, and CI effectively inhibits transcription from the major phage promoters p_L and p_R and thus also expression of phage genes essential for the lytic development. Summing up our observations, we found that the 24B_1 molecule, which under many aspects resembles eukaryotic microRNAs, plays an important role in the phage decision between two alternative pathways: "lysis vs lysogeny" and indirectly stimulates the lysogenic development of Φ24B phage in *E. coli* bacteria. Based on this significant discovery, at **paper No. 9**, we initiated considerations about the occurrence and role of microRNA type molecules in prokaryotes, as little attention has been given to them so far. Surprisingly, some scientists even deny the existence of such a small prokaryotic RNAs. Analyzing the current state of knowledge, we found previous reports indicating the occurrence of several microRNA-size molecules of bacterial origin. Functions of these molecules were unknown, however the presence of longer precursor transcripts was confirmed in several cases. On this basis, we suggested that prokaryotic microRNA-size molecules undergo a maturation process analogous to eukaryotic microRNAs. Importantly, the mechanism of this processing has not been recognized yet. However, considering the NGS results and the fact that 24B_1 molecule was identified in only one form, we suggested that the cleavage may occur in a specific site. At the same time, we drew attention to reports confirming the existence of bacterial microRNA-size molecules in the outer membrane vesicles and their inhibitory effect on the synthesis of some cytokines and the host's immune system. We noticed another

analogy to eukaryotic microRNAs, which may also be transported in small vesicles, named exosomes. Based on these observations, we suggested the recognition of prokaryotic microRNA-size molecules as another (next to *cis*- and *trans*-encoded small RNAs) group of regulatory RNAs operating in prokaryotic cells. This hypothesis seems to be highly probable in the context of temperate viruses attacking bacterial cells, such as the considered here Stx phages that may develop by both lysogenic and lytic pathway. This is due to the fact that microRNA molecules have been identified and are well-known in viruses infecting eukaryotic cells, mainly herpesviruses (Pfeffer *et al.* 2004). Similarly to Stx phages, these eukaryotic viruses also undergo both latent and lytic cycles and may be even related to them (Baker *et al.* 2005). In turn, microRNAs of herpesviruses play an extremely important role at the stage of virus switch from one cycle to another, and a lot of them (similarly to the 24B_1 molecule) favor the viral latency. In addition to this, these molecules target several cellular pathways, in particular those related to the host immune response and cell cycle control (Gray, 2015; Piedade *et al.* 2016). On this basis, we speculate that the analogous to herpesviruses, microRNA-dependent control of virus switch between latent and lytic development, may occur also in Stx phages. Understanding the basics of this mechanism will be our next scientific goal. We will carry out these research within three projects. In one of them I participate as a co-author and the key-investigator. This project is funded under the Opus 15 competition announced by the National Science Center (No. UMO-2018/29/B/NZ1/00549) and its main aim is to continue research related to molecule 24B_1, in particular to understand the mechanism of its biogenesis and to determine the target sites for this molecule. In the other two projects, I participate as the Principal Investigator (Project Manager). I have obtained funds for their realization in Sonata Bis 8 (No. 2018/30/E/NZ1/00400) and Miniatura-2 competitions (No. 2018/02/X/NZ1/02680) announced by the same institution. In these projects I intend to carry out following research tasks: 1) searching for other such a small RNA molecules involved in the regulation of expression of anti-repressor genes essential for the phage genetic switch and 2) investigating the effect of such molecules on changes in the bacterial metabolism. Undoubtedly, further research on small regulatory RNAs that operate in prokaryotic cells will provide important knowledge, but in the future it also may deliver new therapeutic and diagnostic solutions.

To summarize, the discussed above series of publications, I would like to indicate the most important accomplishments:

1. Paying attention to the difficulties in detecting bacteria that produce Shiga toxins, whose main virulence factors (*stx* genes) are located within the mobile genetic elements.

2. The development of novel procedure for detection of specific well-preserved DNA sequences coding for Shiga toxins, that appears to be specific, simple, rapid and cost effective.

3. Identification of regions highly conserved in the Stx phage genomes and having important role in the phage response to oxidative stress conditions.

4. Demonstration of the inhibitory action of compounds with antioxidant properties on the lytic development of Stx phage in *E. coli* bacteria under oxidative stress conditions.

5. Discovery of the first microRNA type phage molecule, of highly conserved sequence and demonstration of its significant, pro-lysogenic role during Stx phage development in *E. coli* bacteria.

6. Indication of differences in the development of Stx and closely related λ phages and demonstration that, despite the general sequence similarity and its similar organization, in certain regions, these phage genomes differ significantly.

The analysis of the above-mentioned sequence regions, that are well-preserved in the genomes of Stx phages, has a triple significance. On the one hand, we determined the role of the few previously unknown regions of Stx phage genome in the development of this phage. On the other hand, we have indicated the possibilities of using the knowledge about selected regions, in the development of new detection methods and treatment strategies against infections caused by Shiga-toxigenic *E. coli* bacteria. Thirdly, we have proved that knowledge obtained in the case of model λ phage cannot be alaways directly transferred to closely related Stx phages and it is important to verify it in an individual approach.

Supplementary literature

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5. Discussion of other scientific and research achievements.

In addition to the above-mentioned scientific achievement involving 11 thematically related publications, I also conducted research in other areas of molecular biology which resulted in 15 more scientific articles. Among them, 3 encompass results from my master's studies, another 3 were included in my doctoral thesis, and the remaining 9 were published after I obtained my doctoral degree.

My first papers are related to the subject of my master's thesis, which I realized in the Department of Genetics at the Nicolaus Copernicus University in Toruń. This thesis was prepared under the supervision of Prof. UMK, dr hab. Anna Goc, however is based on the results of research carried out in the laboratory of Prof. Oliver Eickelberg at the Justus Liebig University in Giessen (Germany). I joined the group of Prof. Eickelberg in October 2004 (during the first year of supplementary master's studies) and stayed there until the end of September 2005. During my one-year internship at Giessen, I participated in an international program titled "Molecular Biology and Medicine of the Lung" (abbreviated as MBML). This program is intended mainly for PhD students interested in molecular biology and pulmonary medicine. First year of the MBML includes exercises, seminars, lectures and exams in which I took part and which I passed very well as a graduated with a Bachelor's degree. In addition to MBML course, I also carried out research in the laboratory of Prof. Oliver Eickelberg. The aim of my research was the understanding of the molecular mechanisms of two chronic diseases: idiopathic pulmonary fibrosis and pulmonary arterial hypertension (PAH). My research has resulted in master's thesis written in English and entitled "TGFβ receptor determinines progressive fibrosis". Besides, the results of my work are also three articles published in high-rated journals from the JCR list: *Arteriosclerosis, Thrombosis, and Vascular Biology; European Respiratory Journal* and *Circulation.* In our research we used, a quite new at that time an induced by monocrotaline (MCT) rat model of PAH, and the pulmonary smooth muscle cell line (PASMC). Investigating the individual factors belonging to the transforming growth factor β (TGF-β) superfamily, we showed that in the lungs of MCT-treated rodents the expression of type 2 receptor TGFBR-2 and the Acvr1 receptor was significantly reduced at both mRNA and protein levels. Moreover, we observed also the significantly reduced levels of proteins Smad3 and Smad4, but also phospho-Smad2, what we confirmed on the PASMCs, derived from the tested rats. On this basis, we suggested the functional impairment of the TGF-β/Smad2,3 signalling axis in the lungs of the MCT-treated rats, and thus the important role of the TGF-β ligand-dependent pathway in the PAH disease **[ref. II.A.1 Appendix 4]**. In parallel, we analyzed the other signaling pathway dependent on factors belonging to the family of bone morphogenetic proteins (BMPs). In this case, we also observed a significant downregulation of expression of receptors (BMPRIb, BMPRII), and Smads (4, 5, 8 and pSmad1), demonstrating the dysfunction of this pathway in the monocrotaline-induced PAH model **[ref. II.A.2**

Appendix 4]. In the last paper from this period, we proved the co-localization, and the existence of interaction between the BMPRII receptor and the RACK-1 receptor protein, in the lung tissues obtained from patients with PAH. We confirmed this results using MCT-induced rat model of PAH, and showed a significant reduction of the level of gene expression and the amount of RACK-1 protein in the lung homogenates. We have also noted the relation between the RACK1 and the phospho-Smad1 protein and its influence on intensity of PASMCs proliferation. On this basis, we have suggested that the RACK-1 protein may play an important role in the pathogenesis of PAH **[ref. II.A.3 Appendix 4]**. As indicated in the cited articles, some of the discussed results were obtained by us in the frame of project entitled "Pulmotension", financed by the 6th Framework Program of the European Union (No. LSHM-CT-2006-018725).

In 2006, after defense of my master's thesis at the Nicolaus Copernicus University in Toruń, I came to Gdańsk and started doctoral studies at the Faculty of Biology of the University of Gdańsk. I carried out my research at the Department of Molecular Biology under the supervision of Prof. Grzegorz Węgrzyn. The subject of my doctorate also concerned Shiga-toxigenic *E. coli* strains and infecting them Stx bacteriophages, but at that time, I focused mainly on understanding the regulatory mechanism of phage replication. My doctoral dissertation consists of three scientific articles, in which I am the first author and which were published in the JCR journals: *Journal of Molecular Microbiology and Biotechnology*; *Microbiology* and *Foodborne Pathogens and Disease*. In my work I used both, the phage models and phage-derived replicons bearing replication regions of phage genomes, encompassing all genes and regulatory sequences required for the initiation of DNA replication from the unique site called *origin*. In the first paper from that period **[ref. II.A.4 Appendix 4**] we observed that, in general, the replication mechanism of the analyzed Stx phages is similar to that observed in the model λ phage, however, there are important differences (in particular at the level of transcriptional activation of the *origin*) which influence the regulation of this process. We also shown that these differences may be related to the specific nucleotide changes in the sequences of genes coding for phage O and P replication proteins. Hence, we proposed that nucleotide differences influence the O-P interaction and consequently also formation and rearrangement of the replication complex. In addition, we have highlighted the role of the DksA protein in this process which (besides ppGpp) is a second important agent of the bacterial response to starvation conditions. Interestingly, under such conditions we observed the inhibition of Shiga toxin phage-derived plasmids replication. We explained this by DksA-mediated less efficient transcription process from p_R promoter and thus not enough transcriptional activation of the *origin* **[ref. II.A.5, Appendix 4]**. In the next paper **[ref. II.A.6 Appendix 4]**, we confirmed these results and noted inhibition of the Stx phage development under such conditions. In further studies we also demonstrated the inhibitory effect of sodium citrate being the component of irrigation fluid (e.g. Orsalit). Curiously, the addition of glucose (which is also a component of such fluids) reversed the effect caused by sodium citrate. Undoubtedly, the obtained results have cognitive significance, although they also seem to be important from a practical point of view. Perhaps, a modification of the composition of irrigation fluids or

inclusion of the fasting phase during treatment, could relieve or eliminate the symptoms of the infection with Shiga-toxigenic bacteria, and thus prevent complications. In addition to the three discussed publications, the equally important scientific achievement of that period, was my first research grant, co-financed by the European Union from the European Regional Development Fund under the Ventures program of the Foundation for Polish Science (No. Ventures/2009-3/6). The support I received, allowed me to carry out some of the research included in my doctoral dissertation. Besides, at that time I worked as a investigator in two research grants funded by the Ministry of Science and Higher Education (No. N301 122 31/3747 and No. N N301 192439).

After receiving PhD, in June 2012, I continued my research work in the KBM laboratory of Prof. Grzegorz Węgrzyn. In addition to the main filed of my research interest (Stx phages), I also took part in research from other scientific areas. Among others, I participated in research based on traditional approach **[ref. II.A.9, Appendix 4**] as well as metagenomic strategy **[ref. II.A.8 Appendix 4]** used to explore cyanobacteria cultured in laboratory conditions, and originating from the Baltic Sea, as source of new, biologically active compounds with potential application in various industries. As a result of the performed experiments, we proved that extracts made from cyanobacterial cultures constitute a rich source of 1) various enzymes; 2) compounds that either stimulate bacterial growth or have antibacterial properties and also 3) compounds revealing anticancer activity. Importantly, the observed profiles of different activities were specific for individual cyanobacterial strains **[ref. II.A.9 Appendix 4]**. In addition to this, we applied a new approach based on the construction and analysis of metagenomic libraries carrying DNA isolated from cultures and blooms of cyanobacteria occurring in Gdańsk Bay. In effect, we identified bacterial clones characterized by the production of compounds of cyanobacterial origin that stimulate bacterial growth or show antibacterial or even anticancer activity **[ref. II.A.8 Appendix 4]**. These studies were carried out as part of the international research grant MAREX (No. FP7-KBBE-2009-3-2-01-245137), obtained in the 7th Framework Program of the European Union. This work resulted in two original papers published in the JCR journals: *European Journal of Phycology* and *Microbial Cell Factories*. Apart from that, we published also two review papers in *Acta Biochimica Polonica* journal. In these reviews, we performed a thorough analysis of the available metagenomic strategies and examples of their application in the discovery of novel enzymes and drugs in various marine environments **[ref. II.A.7 Appendix 4],** and also a detailed summary of the limitations occurring in metagenomics **[ref. II.A.10. Appendix 4]**.

At the same time, I carried out my own research ideas. I became the Principal Investigator of a project financed by the National Center for Research and Development in the frame of LIDER program (Nr Lider/21/92/L-3/11/NCBR/2012). Thanks to the financial support that I gained, I was employed at the University of Gdańsk as a researcher (not a teacher). As a consequence, I had no obligation to have classes and I did research only. In the frame of this project I managed a team of three PhD students. We carried out research to optimize the detection method described in the section 4c (paper No. 4), in particular to modify

this procedure in order to increase its sensitivity and extend the range of identified pathogens. The applied solutions, allowed us to lower the detection threshold to 0.02 pg of DNA, and to use longer probes (over 25 nucleotides), which additionally improved the specificity of the proposed method. In addition, we increased its range for various pathogens transmitted by ticks, responsible for dangerous diseases such as: Lyme borreliosis, babesiosis, bartonellosis rickettsiosis or anaplasmosis. Due to the frequent problem of co-infections with different tick-borne pathogens, and associated with them diagnostic difficulties, the method proposed by us (characterized by increased specificity and sensitivity, compared to classical PCR tests) may become very useful. Research in this topic resulted in two national patents No. B1 218839 **[ref. II.B.1, Appendix 4]** and No. B1 220906 **[ref. II.B.2, Appendix 4]** and another patent application No. P.419159 **[ref. II.B.3 Appendix 4]**, that was submitted to the Polish Patent Office in 2016, in order to expand protection to the introduced modifications. On this basis, we also filed an international patent application (PCT No. EP17001719) to obtain the protection in other European countries **[ref. II.B.4 Appendix 4]**. In addition, this achievement was qualified for the final group of inventions in the competition titled "EUREKA DGP – Odkrywamy polskie wynalazki". As a consequence of this, an article about our invention was published in Polish journal Dziennik Gazeta Prawna on February 2015 [No. 30 (3923) ROK 21]. The invention was also nominated for the EuroSymbol Innovation Award 2015 and attracted attention of the consultant American company 3G Therapeutics. In effect, the authorities of University of Gdańsk decided to commercialize the results of our studies. I am currently conducting pre-implementation research as part of the UG project "Incubator of Innovation +" financed by MNiSW (No. MNISW/2017/DIR/68/II+). The aim of this task is to validate the effectiveness and specificity of the method on blood and cerebrospinal fluid samples obtained from patients with suspected or diagnosed diseases caused by tick-borne pathogens. Sample are collected thanks to the cooperation between the University of Gdańsk and the Pomeranian Hospitals Co.

In 2016, I joined the project financed by the National Science Center in the frame of Opus 9 competition (No. UMO-2015/17/B/NZ9/01724). As an investigator of this project, I took part in task related to discovering of new bacteriophages in the environmental samples and their functional characteristics. This research resulted in two scientific articles, published in journals from the JCR database: *Scientific Reports* and *Frontiers in Microbiology*. In the first paper **[ref. II.A.13, Appendix 4]**, we made a detailed analysis of 83 new bacteriophage strains isolated from urban sewage. These phages have been characterized by us in terms of their basic features, such as host range, virion morphology, plaque morphology, ability to multiply at different temperatures, and sensitivity to physical and chemical factors. In addition, we sequenced the genomes of 7 selected phages, and underwent extensive bioinformatic analysis of the obtained sequences. When comparing the results for various phages, we decided to characterize one of them in detail. We chose the lytic phage vB-EcoS-95. Analysis of this phage indicated that despite of 74% similarity to the genomic sequence of phage pSf-1, the vB-EcoS-95 bacteriophage presents a different host range and does not infect

Shigella bacteria. In addition, vB-EcoS-95 is characterized by a rapid intracellular development in *E. coli* and the ability to destroy bacterial biofilms **[ref. II.A.15 Appendix 4]**. Research in this topic is still ongoing, in the frame of cooperation with Prof. Alicja Węgrzyn from the Laboratory of Molecular Biology at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Gdańsk. Our experience in biofilm-related research allowed us to start cooperation with Paivi Tammela, PhD and Cristina Durante Cruz, PhD, from the University of Helsinki in Finland, and investigate the impact of new antibacterial compounds on biofilms created by the selected strains of Gram-positive bacteria.

I highly appreciate that working at the Department of Molecular Biology at UG, I have the opportunity to cooperate with scientists from other, also foreign, institutions. Undoubtedly, this is due to the head of the Department, Prof. Grzegorz Węgrzyn, who is a well-known and recognizable scientist worldwide. This cooperation has resulted in several important achievements. Together with Prof. G. Węgrzyn, we were invited by Prof. Luciano Saso from the Sapienza University in Rome (Italy) to write a chapter titled "*In vitro* methods for the evaluation of oxidative stress". This chapter was included in the second volume of book series "Recent advances in analytical techniques" published by Bentham eBooks, in 2018. We prepared the subsection related to various strategies used to assess the level of oxidative stress in bacterial cells **[ref. II.C.1 Appendix 4]**. Active cooperation with Prof. L. Saso started from the paper published by our team in the journal *Oxidative Medicine and Cellular Longevity*. In this paper we showed that the *exo-xis* region plays an important role in the Stx prophages induction and bacterial SOS response under conditions of oxidative stress **[ref. II.A.12 Appendix 4 – described in section 4c]**. Professor Saso was very interested in our reflections on the influence of these conditions on the bacterial pathogenicity, and that's why he encouraged us to analyze provided by him antioxidant compounds (paper No. 10 described in the section 4c).

In effect of the research carried out in the *exo-xis* topic, we have also started the cooperation with Prof. Logan Donaldson from the University of York in Toronto (Canada). Prof. Donaldson is interested in analysis of protein structure and highly familiar with a nuclear magnetic resonance (NMR) spectroscopy. We have contacted the first time in 2013, after he published a paper related to the structure of Ea8.5 protein from the *exo-xis* region of λ phage. As part of our cooperation, we decided to functionally analyze other open reading frames located in this region. In effect, we published together two articles in journals from the JCR list: *Frontiers in Microbiology* and *Viruses*. In the first work, we showed that the *orf63* is a functional gene encoding a protein playing an important role in the development of λ and Stx phages in *E. coli* bacteria, especially at the stage of the lysis vs. lysogenization decision. We observed that the deletion of phage *orf63* caused a significant increase in the efficiency of lysogenization and thus delayed the time and decreased the efficiency of prophage induction, what resulted in turn, in the increased survival of bacteria during phage lytic development under oxidative stress conditions. **[ref. II.A.14 Appendix 4]**. In the second paper, we proved that *orf60a* and *orf61* are also important during induction of Stx prophages with hydrogen peroxide

as their absence resulted in similar to *orf63* effects (paper No. 11 described in the previous section). As a result of our cooperation, Prof. Donaldson invited me to his lab in Canada. I am going there to do the training and carry out a series of experiments using the NMR technique to analyze the metabolic profile of *E. coli* bacteria. I've got funds for this research trip in the Miniatura-2 competition, organized by the National Science Center (No. 2018/02/X/NZ1/02680). In the case of research on *orf61* and *orf61*, in 2018 we also started cooperation with Prof. Andrey Letarov from the Vinogradski Institute of Microbiology at the Russian Academy of Sciences in Moscow. After numerous scientific consultations and his visit in the KBM UG laboratory, we have developed research plans for analyzing the effects of *orf60a* and *orf61* on the process of adsorption of lambdoid phages to bacterial cells.

I have also experienced effective cooperation in our local scientific community. In 2015, together with the team of Prof. Agnieszka Szalewska-Pałasz, we tested the effect of RNA polyadenylation on the development of λ and Stx phages. We showed that under conditions of poly(A) polymerase I (PAP I) deficiency in the cell, the lytic development of these phages after both infection and prophage induction was significantly less efficient than in the wild-type host. As in prokaryotic cells, polyadenylation causes destabilization of RNAs, we have suggested that the observed differences are due to the disturbance of the amount of RNA molecules present in the cell. We speculated that in the absence of the PAPI enzyme, RNAs are not subjected to polyadenylation and thus cannot be effectively degraded. We also paid attention to the phenomenon of polyadenylation of regulatory RNA molecules **[ref. II.A.11 Appendix 4]**.

This last aspect seemed very interesting to me and thus, together with Sylwia Bloch, PhD (then PhD student, who I supervised), we attempted to isolate and identify the polyadenylated RNA molecules essential for the development of Stx phages in *E. coli* bacteria. This attempt was unsuccessful, but this failure prompted us to take a decision, very important for our further research, to look for small microRNA-size molecules in prokaryotic organisms. These searches were successful and in this way we discovered the first such a small phage RNA molecule, 24B_1, which was described in detail in the previous section (paper No. 7). As I mentioned earlier, in effect of research carried out in the context of the 24B_1 molecule, many new questions and research ideas were born. They will be develop by us within two big projects financed from Opus 15 (No. UMO-2018/29/B/NZ1/00549) and Sonata Bis 8 (No. 2018/30/E/NZ1/00400) competitions. The first project is related to 24B_1, and I'm co-author and the main investigator of it. In the second one, I am the Principal Investigator and I'm going search for other analogous RNAs, with particular emphasis on molecules that may regulate the expression of genes coding for anti-repressor proteins. Due to the fact that, in addition to repressors (such as CI protein in Stx phages), anti-repressors are the most important proteins regulating the development of phages in bacteria, the identification and characterization of this type molecules would have high cognitive and potentially also application significance.

To sum up, I'm the co-author of 26 articles published in journals from the JCR list, of which 20 come from my postdoctoral period. Eleven articles (out of these 20) are included in the scientific achievement presented for evaluation. The total Impact Factor of all my publications is **97.8**, while the number of citations is **364**, and the Hirsch Index is **10**. I am also a co-author of two national patents and two additional patent applications, including an international one. My next achievement is the co-authorship of the chapter in the book "Recent advances in analytical techniques". As a Principal Investigator I took over management in 3 research projects (including two for the amount of over 1 million PLN) and 2 scientific activities (Miniatura-2 and Incubator of Innovation +). Besides, I participated also in 8 other projects and developed my skill by participating in numerous trainings and courses related to different techniques such as: RT-qPCR, expression profiling of microRNAs, RNA-Seq etc. I also received numerous awards and scholarships, 16 in total, including Scholarship FNP Start (2013), Scholarship for outstanding young scientists from the Ministry of Science and Higher Education (2015) and honorable mention in the Scientific Awards of Polityka journal (2017).

As part of the popularizing and teaching activities, I presented results of my research at 16 conferences and scientific meetings, including 6 speeches. I also participated in the organization of a scientific conference entitled "4th Congress of Baltic Microbiologists", which took place on 10-12 September 2018, in Gdańsk. In addition, I took part in a few workshops during the Baltic Science Festival organized by the Faculty of Biology at the University of Gdańsk. In 2019, I joined the editorial group of the *Postępy Mikrobiologii* journal. Besides, I have reviewed both original and review scientific papers for international journals from the JCR database: *Acta Biochimica Polonica* (4) *Viruses* (3), *Scientific Reports* (1) and outside this list: *Current Bionanotechnology* (1) and *African Journal of Biotechnology* (1). Apart from that, I also reviewed 4 diploma theses. During my current academic career I took the role of an auxiliary supervisor of 4 PhD students and supervisor of 3 master's and 5 bachelor's students. At the Faculty of Biology UG I teach classes in the following subjects: Molecular Biology and Biotechnology and Molecular Diagnostics.

A detailed list of my scientific, popularizing and teaching activities can be found in the Appendix 4.

B. Nejman-Faleńczyk

(podpis)