Annex 3

WYDZIAŁ BIOLOGII
UNIWERSYTET GDAŃSKI

SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

Dr Barbara Kędzierska

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Gdańsk, 2022

1. **Name:** Barbara Kędzierska

2. **Diplomas, degrees conferred in specific areas of science or arts, including the name of the institution which conferred the degree, year of degree conferment, title of the PhD dissertation**

   29.06.1998 - Master's degree in biology (specialisation: molecular biology); Faculty of Biology, Geography and Oceanology (now Faculty of Biology), University of Gdańsk; thesis supervisor: Dr (now Prof. Dr.) Michał Obuchowski; thesis title: "Genetic analysis of bacteriophage λ CII activator interactions with RNA polymerase of *Escherichia coli* in promoter regions p_I and p_{aQ} "

   12.12.2003 - PhD degree in biology; Faculty of Biology, Geography and Oceanology (now Faculty of Biology), University of Gdańsk; supervisor: Prof. Dr. Grzegorz Węgrzyn; thesis title: "Mechanism of transcription activation by the CII protein of bacteriophage λ". Doctoral thesis defended with honours.

3. **Information on employment in research institutes or faculties/departments or school of Arts**

   01.10.1998 to **30.09.2002** - a doctoral student at the Environmental Doctoral College of the Faculty of Biology, Geography and Oceanology of the University of Gdańsk

   01.05.2000 to **01.08.2000** - trainee at the Laboratory of Molecular Microbiology, University of Sheffield Medical School, UK under an EMBO Short Term Fellowship

   03.06.2002 to **03.09.2002** - trainee at the School of Bioscience, University of Birmingham, UK as part of the FEBS Collaborative Experimental Scholarship for Central & Eastern Europe

   01.10.2002 to **14.10.2006** - assistant in the Department of Molecular Biology, Faculty of Biology, Geography and Oceanology, University of Gdańsk

   12.01.2004 to **12.08.2006** - postdoctoral research assistant at Manchester Interdisciplinary Biocentre, University of Manchester, UK

   15.10.2006 to **31.12.2016** - assistant professor in the Department of Molecular Biology, Faculty of Biology, Geography and Oceanology, and since 2009 in the Faculty of Biology, University of Gdańsk

   09.11.2006 to 30.09.2007 - maternity and parental leave

   03.08.2009 to 01.03.2010 - maternity leave

   01.01.2017 until now - assistant professor in the Department of Molecular Genetics of Bacteria, Faculty of Biology, University of Gdańsk

4. Description of the achievements, set out in art. 219 para 1 point 2 of the Act (a) Title of scientific achievement

The achievement presented for evaluation is a series of **seven** thematically related publications (**5** original papers and **2** review papers), which I have included under a common title:

"Molecular basis of gene expression regulation and specificity between homologous toxin-antitoxin systems derived from *Escherichia coli* and *Enterococcus faecium*"

(b) Publications forming part of the scientific achievement

[1] Kędzierska B, Lian LY, Hayes F*. (**2007**). Toxin-antitoxin regulation: bimodal interaction of YefM-YoeB with paired DNA palindromes exerts transcriptional autorepression. *Nucleic Acids Res*. 35: 325-39.

IF_{2007} =6.954; $MNiSW_{2007}$ =40; according to the latest **MEiN** scoring$_{2021}$ **=200**

[2] Boss L, Labudda L, Węgrzyn G, Hayes F, **Kędzierska B***. (**2013**). The Axe-Txe complex of *Enterococcus faecium* presents a multilayered mode of toxin-antitoxin gene expression regulation. *PLoS One*. 8: e73569**.**

IF_{2013} =3.534; $MNiSW_{2013}$ =40; according to the latest **MEiN** scoring $_{2021}$ **=100**

[3] Połom D, Boss L, Węgrzyn G, Hayes F, **Kędzierska B***. (**2013**). Amino acid residues crucial for specificity of toxin-antitoxin interactions in the homologous Axe-Txe and YefM-YoeB complexes. *FEBS J*. 280: 5906-18.

IF_{2013} =3.986; $MNiSW_{2013}$ =30; according to the latest **MEiN** scoring $_{2021}$ **=100**

[4] Hayes F*, **Kędzierska B***. (**2014**). Regulating toxin-antitoxin expression: controlled detonation of intracellular molecular timebombs. *Toxins* (Basel) 6: 337-58.

IF_{2014} =3.229; $MNiSW_{2014}$ =30; according to the latest **MEiN** scoring $_{2021}$ **=100**

[5] Kędzierska B*, Hayes F*. (**2016**). Emerging Roles of Toxin-Antitoxin Modules in Bacterial Pathogenesis. *Molecules*. 2: pii: E790.

IF_{2016} =2.861; $MNiSW_{2016}$ =30; according to the latest **MEiN** scoring $_{2021}$ **=140**

[6] Kędzierska B*, Potrykus K, Szalewska-Pałasz A, Wodzikowska B. (**2020**). Insights into Transcriptional Repression of the Homologous Toxin-Antitoxin Cassettes *yefM-yoeB* and *axe-txe*. *Int. J. Mol. Sci.* 21, 9062.

IF_{2020} = 4.556; $MNiSW_{2020}$ =140

[7] Kędzierska B*, Potrykus K. (**2021**). Minigene as a Novel Regulatory Element in Toxin-Antitoxin Systems. *Int. J. Mol. Sci.* 22, 13389.

IF_{2021} = 6,208; $MNiSW_{2021}$ =140

* - Corresponding author

The impact factor (IF) and scores of the above publications are given according to the year of publication and according to the latest MEiN scoring.

The total impact factor (**IF**) of the above publications according to the year of publication is **31.328**

The number of citations of these papers according to the Google Scholar database is **330**; according to Web of Science is **226**; according to the Scopus database it is **245**, of which **225** without self-citation (data as of 14.11.2022)

The statements of the co-authors of the publications specifying the individual contribution of each author to the individual publications can be found in **Appendix 5**. The statements of the postdoctoral researcher concerning the contribution to the completed works can be found in **Appendix 4, section I2.**

(c) Discussion of the scientific objective of the above work and the results achieved

The scientific objective of the above-mentioned work was to describe the molecular mechanisms regulating the expression and specificity of the homologous toxin-antitoxin (TA) systems *yefM-yoeB* and *axe-txe*, derived from the *Escherichia coli* chromosome and the *Enterococcus faecium* pRUM plasmid, respectively. Furthermore, it was important to place the results obtained in a broader context related to the different regulatory strategies of TA modules and their potential role in the pathogenesis of bacterial diseases.

Toxin-antitoxin systems are small modules, usually composed of a pair of overlapping genes encoding a toxin and its antidote. They are widely distributed on plasmids and chromosomes of most bacterial species and some *Archaea*, often in multiple copies (Yamaguchi et al., 2011). The toxin is sometimes referred to as an intracellular molecular timebomb, as its release from the complex with the antitoxin results in growth inhibition or even cell death. Hence, the expression of both genes must be properly balanced, as too much toxin can be lethal to the cell. The vast majority of known toxins attack the translation process, acting as endoribonucleases that cut free or ribosome-bound mRNA, but there are also those that are inhibitors of the replication process, peptidoglycan synthesis or cause disintegration of the cell membrane. Due to the nature and mode of action of antitoxin, eight types of TA systems have so far been distinguished (Jurėnas et al., 2022; Singh et al., 2021). Type II modules, in which both the toxin and its antidote are proteins and the abrogation of toxicity occurs after physical blocking of the toxin's active centre by the antitoxin, are the best known and most widely distributed among bacteria. Most are characterised by a similar structure and mode of expression regulation. They consist of a pair of overlapping genes forming an operon, the first encoding a cellular protease-sensitive (Lon or Clp) antidote and the second a stable poison. The strong and specific interactions between the toxin and its antidote, as well as the tight regulation of the expression of these genes, are hallmarks of all known TA type II modules. The controlled

production of toxin and antitoxin in these systems is usually achieved by negative autoregulation at the transcriptional level - the antitoxin binds with its N-terminal part to palindromic sequences within the promoter, thus being directly responsible for repression, while the C-terminal domain binds the toxin, which acts as a corepressor increasing the affinity and stability of the whole complex (Kedzierska & Hayes, 2016). Additionally, it has been shown in some systems that cooperative binding of the complex to DNA only occurs if both proteins are present in appropriate stoichiometric amounts, as excess toxin causes derepression of the operon by releasing the complex from the operator (Cataudella et al., 2012). The first plasmid-based TA modules were described in the 1980s, while only a decade later the first chromosomal cassettes of this type were identified (Hayes & Van Melderen, 2011). The role of plasmid modules is not in doubt and is the stable maintenance of these genetic elements in the bacterial cell population. It is associated with an addiction mechanism that involves the post-segregation elimination of cells that have lost the plasmid. In progeny cells that have not inherited the TA module, the degradation of the proteolysis-prone antitoxin and the absence of its *de novo* synthesis, results in the release of a stable toxin that, by combining with its specific cellular target, leads to cell death or to the inhibition of its metabolic processes. In this way, cells become 'addicted' to TA modules located on plasmids. Many plasmid addiction genes have their homologues on bacterial chromosomes, but their function is still not entirely clear and is the subject of extensive debate in the scientific community. It is thought that they may have a role in stabilising mobile genomic elements, act as an anti-addiction to plasmid DNA, protect against bacteriophage infection, enable cells to adapt their metabolism under changing environmental conditions, but their role in biofilm formation, persister cells and bacterial virulence has also been indicated (Jurėnas et al., 2022; Singh et al., 2021).

One of the chromosomal systems discovered in the *E. coli* genome is the *yefM-yoeB* cassette (Pomerantsev et al., 2001). It has been shown that this module functions as an active toxin-antitoxin system and that its homologues are widespread in the genomes and on plasmids of different bacterial species (Grady & Hayes, 2003). My task during post-doctoral training in the laboratory of Dr. Finbarr Hayes at the University of Manchester in the UK was to learn more about the molecular basis of the regulation of gene expression of this cassette, and the results of this research are summarised in **publication 1**. I began my work by determining the transcription start site of the *yefM-yoeB* operon genes, which helped me identify the '-10' and '-35' boxes of the promoter, which we named p_{yy} , and the start codon for the YefM antitoxin. The results I obtained revealed that **for the experiments published in the meantime, the 10 amino acids longer YefM antitoxin was erroneously used, with as a start codon the ATG lying within the '-10' hexamer of the promoter I had designated** (Cherny et al., 2005; Cherny & Gazit, 2004). Both of these papers described YefM as a naturally unfolded protein that only becomes structured upon binding to the YoeB toxin. In contrast, my studies have shown that YefM possesses significant α-helices and β-sheets and tertiary structures, and that the unstructured region is located only at the C-terminus of this protein and indeed folds upon toxin binding. I also showed that the antitoxin mainly exists as a dimer and the complex as a trimer $YefM_2YoeB$, which was consistent with the crystal structure published at the time (Kamada & Hanaoka, 2005). Then, using *in vivo* as well as *in vitro* techniques, I investigated the function of the p_{yy} promoter - alone and in the presence of YefM and the YefM-YoeB complex. These results showed that antitoxin is a weak repressor of its own promoter, and that

toxin acts as a specific corepressor to enhance and stabilise this interaction. Furthermore, within the p_{yy} promoter, I identified sequences to which antitoxin binds. These are two palindromic repeats with a common TGTACA core, whose centres are separated by 12 nucleotides. One of these encompasses the '-10' region, while the other surrounds the transcription start site of this promoter. I have also shown that the first of these sites is recognised by the antitoxin first and its presence is crucial for the cooperative binding of this protein (or TA complex) to the second site. In summary, **my studies show that the *E. coli* chromosomal *yefM-yoeB* module is regulated in a manner typical of most type II TA systems and is negatively autoregulated at the level of transcription initiation, where two YefM$_2$YoeB complexes bind in the p_{yy} promoter region thereby blocking the RNA polymerase (RNAP) site**. In this way, the concentration of the toxin is controlled and maintained at a safe level for the cell. We also analysed the sequences of *yefM-yoeB* cassette homologues from different bacterial species and found that many, but not all, of them have a similar arrangement of inverted TGTACA repeats.

One such TA system homologous to *yefM-yoeB* is the *axe-txe* module, which was identified on a multidrug-resistant pRUM plasmid from *E. faecium* in the laboratory of Dr. Finbarr Hayes (Grady & Hayes, 2003). I was interested in whether the gene regulation of this operon looks similar to the chromosomal *yefM-yoeB* cassette of *E. coli.* Previous experiments have shown that the *axe-txe* module functions efficiently not only in enterococci, but also in evolutionarily distant bacterial species such as *E. coli* or *Bacillus sp*. (Grady & Hayes, 2003). Thus, due to the limited access, number and sensitivity of genetic tools for enterococci, we performed our experiments in cells of a model organism such as *Escherichia coli* (**publication 2**). The research for this and for subsequent papers was mostly developed under a grant from the Polish Ministry of Science and Higher Education (no. 519/B/P01/2009/36), of which I was the principal investigator. Our results showed that the *axe-txe* operon differs markedly in complexity from previously described TA modules, including its homolog *yefM-yoeB.* We identified as many as three transcripts arising from this operon. The antitoxin and toxin genes are co-expressed from the p_{at} promoter, which is extremely strong and is repressed by the Axe-Txe complex, but only to a certain extent. We have shown that an additional portion of the toxin arises from a promoter lying within the antitoxin gene, the p_{axe} promoter. This promoter appears to act constitutively, as neither the proteins of the module under study, nor any of the cellular proteins, affect its activity. Inactivation of p_{axe} disrupts the proper balance between toxin and antitoxin, so that, as we have shown, the system loses its ability to stably maintain the plasmid in the bacterial population. We also detected a third transcript, this time arising from a promoter inside the *txe* gene, in a reverse cassette orientation, which we have named p_{txe}, and which appears to represent another regulatory element. This transcript is complementary to transcripts from the p_{at} and p_{axe} promoters. Since non-coding RNAs can significantly affect the regulation of gene expression, such a transcript may downregulate the production of Txe and/or Axe proteins by blocking translation or by colliding transcriptional complexes producing sense and antisense RNAs. An additional regulatory element also appears to be the termination hairpin identified in this work located just downstream of the toxin gene. We observed that bacteria carrying a plasmid with an *axe-txe* cassette lacking this sequence show significant growth inhibition. It seems to us that the *axe-txe* structure may be recognised by RNases potentially associated with the degradosome. This would result in reduced transcript stability and lead to less toxin protein. Both the mode of action of the p_{txe} promoter-derived

transcript and the termination hairpin require experimental verification; however, the results presented in this work clearly show that **the *axe-txe* system, although homologous to *yefM-yoeB*, is regulated in a complex and multifaceted manner**, at least in *E. coli* cells.

Moreover, when I analysed the sequence of the 114-nucleotide leader segment of the transcript arising from the p_{axe} promoter I noticed three potential translation start codons (two ATGs and one GTG), all in the same reading frame with the TAA stop codon. These triplets are also all in the same reading frame as the ATG codon for the Txe toxin. This discovery prompted me to test whether their presence somehow affects *txe* expression (**publication 7**). The immediate inspiration for the experimental work was a publication from the laboratory of Prof. Steve Busby, with whom I had done one of my internships many years earlier (EMBO fellowship at the University of Birmingham, UK, in 2002). This work concerns a two-codon minigene identified in the transcript of the *LEE1* operon of an enterohaemorrhagic *E. coli* (EHEC) strain, the presence of which appeared to directly affect the activity of the downstream gene *ler* (Islam et al., 2012). In recent years, there have also been a number of bioinformatics analyses, as well as transcriptomic and ribosome profiling, which have shown that small open reading frames (ORFs) are abundantly present in bacterial genomes, including in non-translated regions located at the 5' ends of many genes (5'-untranslated regions - 5'-UTR). Nevertheless, to date, the mechanisms of action of only a few mini-ORFs have been described. The presence of a mini-ORF can have a positive or negative effect on the expression of the downstream gene and can modulate it in various ways. In my work, I applied genetic studies using translational fusions with the *lacZ* reporter gene. I first showed that the ATG2 codon (together with the surrounding nucleotide sequence) exhibits strong translational potential and is a start codon for a two-codon minigene. I then showed that inactivating this mini-ORF by introducing mutations resulted in a more than eightfold decrease in reporter gene expression. Interestingly, complete deletion of the leader region had the opposite effect. Such a result may seem illogical, however, it points to a likely mechanism by which the minigene regulates *txe* expression. Here I was helped by a paper showing that the mRNA for the *esp* gene of the *LEE4* operon of EHEC cells is protected from attack by ribonuclease E (RNase E) by a ribosome bound to a six-codon minigene present in the leader region (Lodato et al., 2012). The leader region of the transcript from the p_{axe} promoter contains at least two AU nucleotide-rich sequences located upstream of the ATG1 and ATG2 codons. Such sequences are potentially recognised by the ribosomal S1protein, an essential component of the translation machinery responsible for mRNA selection and enhancing the efficiency of this process, but also by RNase E, which also has a so-called S1 domain responsible for substrate recognition. Thus, there is probably competition between the ribosome and RNase E for the site of action. Therefore, it seems reasonable to assume that the binding of the ribosome protects the leader segment of the transcript emerging from the p_{axe} promoter from degradation by ribonuclease, thus increasing its stability and translational efficiency. This hypothetical mechanism seems plausible, but needs experimental confirmation. In the work described here, I have also shown that the minigene works only *in cis*, and that its overexpression is not toxic to *E. coli* cells, as is the case with some synthetic minigenes (Cruz-Vera et al., 2003). In the remainder of this work, I set out to answer two more general and universal questions: 1) does it matter which reading frame the minigene and the gene it regulates are in? 2) does the distance between the minigene and the gene regulated by it matter? Our studies have shown that a mini-ORF can be

located in any of the three reading frames relative to the downstream gene, but then acts with different efficiency. In contrast, the corresponding distance between both cistrons is a more crucial parameter and a change of even one codon weakens this effect. I suspect that this is related to the mechanism of so-called translational coupling, whereby genes are translated by a single ribosomal complex when the distance between them is sufficiently small. We showed that gradually increasing this distance led to a slow loss of the minigene's effect on reporter gene expression and inhibition of its activity. This happens when the distance is too large for translation coupling and too small for two separate ribosomal complexes to fit. Thus, it appears that, **in addition to protecting the mRNA from degradation by the ribonuclease, translational coupling is an additional mechanism by which the minigene in the leader segment of the p_{axe} transcript affects Txe toxin expression**. Furthermore**, this is the first case described in which a minigene regulates toxin expression in a toxin-antitoxin system**. Using a computer programme developed by my student, we analysed 140 antitoxins from several different families of TA systems for the presence of two-codon ORFs and among them we identified 17 containing minigenes. This shows that such genetic elements may be more widely distributed as a regulatory mechanism of toxin-antitoxin modules.

The aim of my subsequent work was to understand the mechanisms determining the strict specificity of the proteins of the *yefM-yoeB* and *axe-txe* systems towards their biological partners (**publication 3**). Phylogenetic studies show that proteins of distantly related TA systems usually do not exhibit cross-talk interactions, whereas proteins of homologous close modules are often able to interact also with their non-partners (De Bast et al., 2008). Given the hazardous nature of toxins to the bacterial cell and the fact that different toxin-antitoxin cassettes can co-occur in a single genome in multiple copies, whether on chromosomes, plasmids or bacteriophages, it seems crucial for them to avoid non-specific, random cross-reactions. As the crystal structure of the YefM-YoeB complex was already known (Kamada & Hanaoka, 2005), we performed homology modelling of the Axe-Txe complex based on it, in collaboration with Dr Anna Czerwoniec of VitaInSilica. Due to the relatively high identity of the amino acid sequence (25% for antitoxin and 50% for toxin) and the high similarity of the obtained Axe-Txe model and the tertiary structure of YefM-YoeB, I decided to identify the determinants of specificity and to select the amino acid residues crucial for the interaction between the proteins of these TA complexes. Using a bacterial two-hybrid system and toxicity rescue experiments, we confirmed that the interactions of the two natural complexes are highly specific and virtually no cross interactions were observed. Analysis of the amino acid sequences and structures of both complexes allowed us to select amino acid residues potentially determining differences in the binding domains of toxin and antitoxin proteins. Further experiments using mutants at these sites enabled the identification of an Asp83Tyr mutant in Txe that exhibited an efficient interaction of this toxin with the YefM antitoxin and showed that the protein complex so formed was an efficient repressor of the p_{yy} promoter. The Tyr82 residue in YeoB, which corresponds to Asp83 in Txe, interacts with Tyr53 of YefM. The reciprocal arrangement of the aromatic rings in these side chains is stabilised by the hydrogen bonds of each hydroxyl group to the carbonyl groups in the opposite chain. Hence, the Asp83Tyr substitution in Txe sufficiently makes this protein similar to YoeB and enables its interaction with the YefM antitoxin. Furthermore, our analyses showed that the interactions between Axe and Txe proteins are of a different nature. The positions Tyr82 in YoeB and Tyr53

in YefM correspond to the negatively charged Asp83 residue in Txe and the positively charged Arg54 residue in Axe. Thus, **the different nature of the key physicochemical interactions at these positions - ionic interactions in Axe-Txe *versus* the corresponding arrangement of aromatic rings in YefM-YoeB - is likely responsible for the lack of cross-protein interactions between the two systems**. These conclusions are supported by my analysis of the protein sequences of the *yefM-yoeB* cassette homologues from different bacterial species and the available experimental data on the presence or absence of homologous interactions between them. Well, it was shown that the proteins of this cassette from *E. coli* and *Streptococcus pneumoniae* do not interact, and a comparison of the sequences of the two complexes showed that YY_{Spn} is more similar to Axe-Txe than to YY_{Ecoli}, in that Asp83 in Txe and Arg54 in Axe are located at corresponding positions in YY_{Spn} (Nieto et al., 2007). In contrast, another paper demonstrated that YefM from *Mycobacterium tuberculosis* is able to neutralise the toxicity caused by the overproduction of YY_{Ecoli} (Kumar et al., 2008). The proteins of both of these complexes also have a high degree of sequence homology and, in addition, the YY_{Mtub} proteins have tyrosine residues at their respective sites, as does YY_{Ecoli}. In summary, once the complex is formed, the amino acid residues responsible for YoeB toxicity are obscured by the YefM antitoxin. The Glu46, Arg65, His83 and Tyr83 residues are responsible for the toxicity and are conserved in the YoeB homologues. Binding of the YefM antitoxin forces a conformational change in the last three amino acids of YoeB - Tyr82, His83 and Tyr84, which destabilises the catalytic centre of this endoribonuclease (Kamada & Hanaoka, 2005). The YefM residues responsible for this change are Glu50, Tyr53, Ser57, Asn60 and Arg63. Among these, only Tyr53 and Asn60 differ between YefM and Axe. Thus, the only pair of amino acid residues that interact and have significant physico-chemical differences are Tyr82 in YoeB and Tyr53 in YefM. Consequently, these residues are crucial for the specificity of homologous but non-interacting toxins and antitoxins that have non-corresponding amino acid residues at these positions.

The idea for my next paper arose from the observation that, despite the similar structure of the promoter regions and protein-binding operator sequences of the two TA complexes, the transcriptional activity of the p_{yy} and p_{at} promoters and their level of repression are significantly different. While the p_{yy} promoter is weaker and is completely inhibited by the YefM-YoeB complex, the p_{at} promoter was found to be extremely strong and only partially inhibited by Axe-Txe proteins (**publications 1 and 2**). I therefore decided to determine the molecular basis of these differences (**publication 6**). We first showed that the differences in the strength of the two promoters are mainly due to differences in the sequence of the '-35' hexamer of the two promoters. We then showed that binding of antitoxin as well as TA complexes to their own and homologous operator occurs at similar levels. This is not surprising since both operator regions show high similarity in the main repressor binding sites. In both cases, these are two pairs of inverted repeats with an identical core (5'-TGTACA-3') whose centres are separated by 12 nucleotides (**publication 1**). However, during a close analysis of both of these sequences, I noticed that although both palindromes have an almost identical nucleotide sequence, they are aligned differently with respect to the core promoter elements. This observation led us to speculate that perhaps **a different step in the transcription initiation process is disrupted in each of these cases**. The main repressor binding site to the p_{yy} promoter covers the '-10' box, while the second palindrome covers the transcription start site from this promoter. This

arrangement of the operator sequence suggests the formation of a steric hindrance to RNA polymerase binding, which we confirmed experimentally. In contrast, the main repressor binding site in the p_{at} promoter region is located within the linker between the '-10' and '-35' boxes, while the second palindrome partially encompasses the '-10' hexamer. This arrangement of the operator elements potentially allows simultaneous binding of the RNA polymerase and the repressor, and inhibition of transcription initiation is likely to occur downstream of this process. In our experiments, we confirmed that simultaneous binding of the repressor complex and RNAP is possible to the p_{at} promoter sequence. To the best of our knowledge, in all cases described so far in which the protein bound to the linker sequence, there was a blockage of the open complex formation step during transcription initiation. This is because, to effectively open the promoter, the physical deformations that cause conformational changes within the linker occur in parallel with the disruption of hydrogen bonds in the '-10' box (Sztiller-Sikorska et al., 2011). Thus, any protein binding to this region of DNA must block the formation of the open complex. Negative autoregulation at the transcription initiation step is a feature of most operons of type II TA systems, however, to date the exact step in this multi-step process that is blocked by binding of the repressor complex had not been studied for any of them. Another element that we identified in the work for this publication is **an additional promoter located 40 nucleotides upstream of the p_{at} promoter, which we have named p_{at2}**. According to our experiments, it contributes positively to the total expression of *axe-txe* genes, and since it is not regulated by the system proteins, transcription from this promoter masks the level of repression of the p_{at} promoter. This explains the need for additional regulatory factors to control the correct balance of toxin and antitoxin production. Thus, the presence of elements such as internal promoters within the *axe* and *txe* genes, as well as a terminator hairpin downstream of *txe*, or the minigene I recently identified in the leader segment of a p_{axe} promoter-derived transcript ensure the proper function of this cassette.

The next two publications I included in my scientific achievement are review papers (**publications 4 and 5**). The first of these (**publication 4**) deals with the **different regulatory strategies that have evolved in type II toxin-antitoxin systems to control the appropriate levels of both proteins in the bacterial cell**. The idea for this work arose from the need to place the results I obtained on the regulation of the expression of the *yefM-yoeB* and *axe-txe* operons in a broader context and to confront them with data obtained in studies of other TA systems. The vast majority of TA type II modules known to date use a negative autoregulation mechanism at the level of transcription initiation to regulate the expression of their genes. In a typical system, the poison and antitoxin genes are expressed from a single promoter within which the operator sequences are located. Typically, the antitoxin binds directly to the operator site partially inhibiting transcription from its own promoter, with the toxin acting as a specific corepressor to enhance and stabilise this interaction. However, a careful analysis of the literature data has provided us with many examples of variations on this theme. We have presented systems in which additional regulatory elements (genes, promoters) have been identified, as well as those in which the toxin or a completely different protein acts as a repressor of the whole system. We also described modules that are not autoregulated at all and examples of TA proteins that regulate the expression of various cellular genes. In addition, we have analysed feasible and already published strategies for the artificial induction of a toxin,

including by disrupting the regulation of its expression, in order to develop potential methods to combat bacterial infections by 'suicide from within'.

The second review paper is on the role of toxin-antitoxin systems in the pathogenesis of bacterial diseases (**publication 5**). In it, we summarised the current knowledge of the **molecular mechanisms by which TA systems** (including YefM-YoeB from *E. coli* and its homologues from pathogenic bacterial species) **are directly and actively involved in bacterial virulence, persister cell production and biofilm formation**. We have shown the complex and multifaceted protein networks coordinating these processes and the role of TA modules in these systems. Although it is now believed by some scientists that the role of TA systems in persister cell formation is not reliably documented (Song & Wood, 2020), but nevertheless there are indications that such a function may potentially exist.

I consider my most important achievements presented in these publications to be:

- to learn about and describe the mechanism of gene expression regulation of the *yefM-yoeB* system;
- demonstrating that YefM antitoxin is not a natively unfolded protein, as previously thought, but has significant secondary and tertiary structures;
- demonstrating that the regulation of *axe-txe* gene expression (studied in *E. coli* cells) is very complex, which distinguishes this system from others described so far;
- discovering minigene as a novel regulatory factor in toxin-antitoxin systems and proposing a potential mechanism of action of this genetic element on Txe toxin expression;
- to elucidate the mechanism determining the high specificity of the two modules and the lack of cross-interactions between the protein partners; we showed that it is related to the different nature of the physicochemical interactions between the key amino acid residues of the poison and antidote proteins (ionic interactions between Asp83-Txe and Arg54-Axe versus the corresponding arrangement of the aromatic rings in Tyr82-YoeB and Tyr53-YefM);
- demonstrating that for the different efficiency of transcription inhibition from the p_{yy} and p_{at} promoters different distribution of repressor binding sequences relative to the main promoter elements is responsible - other steps in this process are blocked for each of these promoters (p_{yy} - blocking the RNAP site; p_{at} - preventing the formation of an open complex);
- a detailed review and analysis of the various mechanisms regulating gene expression of type II TA systems;
- to show the complex and multifaceted networks of protein interactions coordinating virulence, persister cell and biofilm formation and the role of TA modules in these processes.

Supplementary literature (includes only the most important items):

Cataudella, I., Trusina, A., Sneppen, K., Gerdes, K., & Mitarai, N. (2012). Conditional cooperativity in toxin-antitoxin regulation prevents random toxin activation and promotes fast translational recovery. *Nucleic Acids Research*, *40*(14), 6424-6434.

https://doi.org/10.1093/nar/gks297

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d). Current research and future scientific plans

The studies described above have shown that the regulation of *yefM-yoeB* gene expression is typical of most known TA type II systems and involves negative autoregulation at the transcription initiation stage, whereas in the case of the *axe-txe* module it is very complex, which distinguishes this system from others known to date. The presence of a very strong p_{at} promoter has made it possible to see an extremely complex mechanism controlling the expression of this operon, where, in addition to the typical negative autoregulation, we have identified additional regulatory elements inside the cassette. Nevertheless, further studies are needed to understand the exact mechanism regulating the expression of these genes and the interplay of all existing elements of this complex system.

Currently, I am continuing my research to elucidate the mechanism of action of a terminating hairpin located downstream of the *txe* gene, an antisense transcript from the p_{txe} promoter and a minigene identified in the leader segment of the p_{axe} promoter transcript. Bacterial ribonucleases play a potential role in the function of all these genetic elements. In addition, some time ago I started experiments aimed at clarifying how the *axe-txe* cassette is regulated in its natural host, enterococci. Admittedly, the *lacZ* gene reporter system in the pTCVlac plasmid, which I have used to date and which is practically the only one used in the study of enterococci, has only allowed me to see the strongest element, which is the p_{at} promoter, thus I have started to work on developing a *lux* gene-based system, which should be

more sensitive. I have also purified an RNA polymerase derived from these bacteria, enabling me to perform the necessary experiments *in vitro*. In my first *in vitro* transcription experiments with this RNAP, I was able to show all three transcripts arising from the promoters I identified earlier within the *axe-txe* cassette. I believe that the *axe-txe* module provides an excellent model for studying the regulation of TA gene expression, but can also provide valuable insights into the various strategies used by bacteria to precisely control gene expression, in general.

One such regulatory element that has interested me the most is minigenes. I intend to devote my next project to them, for which I will apply to NCN for funding. Bioinformatics data show that small open reading frames are widespread in bacterial genomes, but few have been described to date. There are only a few documented examples in the literature of minigenes affecting the expression of another cistron, and only in one case has a mechanism for this been proposed. Consequently, I see great potential for the future scientific development in this field.

5. Presentation of significant scientific or artistic activity carried out at more than one university, scientific or cultural institution, especially at foreign institutions

In **1993** I started my five-year MSc studies at the Faculty of Biology, Geography and Oceanology, University of Gdańsk. I carried out my thesis in the Department of Molecular Biology headed by Prof. Dr. Grzegorz Węgrzyn, under the direct supervision of Dr. (now Prof. Dr.) Michał Obuchowski. At that time, one of the main research streams in the Department was concerned with the molecular mechanisms determining the development of bacteriophage λ. After infecting host cells, phage λ must choose one of two alternative developmental pathways - lysis or lysogeny. Lytic development leads to the production of progeny virions and their release after lysis of the bacterial cells, while in the lysogenic cycle the phage genome integrates into the host chromosome taking the form of a prophage. The decision on which of these pathways to choose is crucial for virus development and depends on the activity of the CI, CII and CIII proteins. The CI protein is a repressor of the main lytic promoters - p_R and p_L, but on the other hand it is an activator of its own promoter - p_M. Just after infection of the bacterial cell, the p_M promoter is inactive and the p_E promoter is responsible for the early expression of the *cI* gene. It is, together with two other promoters - p_I and p_{aQ} , activated by the CII protein. The p_I promoter is responsible for integrase expression, while the p_{aQ} promoter produces an antisense transcript to the gene encoding the anti-terminator protein Q. The CII protein is rapidly degraded by the cellular protease FtsH (HflB), whose inhibitor is the CIII protein, so its activity indirectly stabilises CII.

The idea for my research arose from previously published results showing that bacteriophage λ cannot enter the lysogenic cycle in the *Escherichia coli rpoA341* mutant. This mutation causes a single amino acid change of K271E in the C-terminal domain of the α-subunit (αCTD) of bacterial RNA polymerase (RNAP), resulting in a defect in transcription activation by the phage CI and CII proteins. We first set out to investigate the effect of the aforementioned mutation on the efficiency of transcription activation from CII protein-dependent promoters via transcriptional fusions. This research resulted in my thesis entitled "Genetic analysis of the interactions of the CII activator of bacteriophage λ with the RNA polymerase of *Escherichia coli* in the promoter regions p_I and p_{aQ} ", which was written in **1998,** and its results were also presented by me at the XXIV Congress of the Polish Biochemical Society in Bialystok (**Appendix 4, point 7a [1]**). In addition, part of the results formed part of

a paper published in *Acta Biochimica Polonica* in the same year, in which we showed that the mechanism of transcription activation from all three CII protein-dependent promoters must be different, due to the fact that each showed a different degree of activation in the *rpoA341* mutant - the activity of the p_E promoter was most impaired, the p_I promoter was shown to be partially active, while the activity of the p_{aQ} promoter was almost unaffected (**Appendix 4, paragraph 4a [1]**).

After graduation, in **1998**, I became a PhD student of the Environmental Doctoral Programme at the Faculty of Biology, Geography and Oceanology, University of Gdańsk. As part of my PhD thesis, under the supervision of Prof. Dr. Grzegorz Węgrzyn, I continued my research on the role of the α subunit of bacterial RNA polymerase in the activation of transcription by CI and CII proteins. Thanks to the collaboration established by my Promoter with Dr. Mark Thomas from the University of Sheffield Medical School in the UK, we obtained a collection of plasmids containing a complete alanine scan of the C-terminal domain of the α-subunit of RNAP. With this, in *in vivo* studies using transcriptional fusions of the promoter under study with the *lacZ* reporter gene, we were able to target the amino acid residues in αCTD responsible for interacting with the CI protein upon activation of the p_M promoter and with the CII protein upon activation of the p_E promoter. However, to confirm that the results obtained show direct interactions between these proteins there was a need to perform *in vitro* studies. To this end, I undertook **two three-month research interships in the UK**, during which I gained experience in a variety of techniques enabling me to study the transcription process *in vitro*. For the first of these I went to Dr. Mark Thomas at the University of Sheffield Medical School in **2000** as part of an **EMBO Short Term Fellowship** awarded to me. While there, I cloned a dozen or so αCTD alanine variants, selected from my earlier studies, into an expression vector allowing purification of these proteins on a nickel column. Meanwhile, I prepared the remaining RNAP subunits in the form of inclusion bodies and then reconstituted the whole holoenzymes with the previously purified α-subunit variants. I also performed the first trial transcription reactions *in vitro* to test the performance of the purified RNAPs and to optimise the reaction conditions, which I have completed in-country. The results obtained in the *in vivo* and *in vitro* experiments appeared to be completely consistent, however, further detailed studies were needed to precisely identify the sites of interaction of αCTD and the activator protein with the promoter region. Therefore, for my second internship, in **2002**, I went to the laboratory of Prof. Steve Busby at the University of Birmingham. Professor Busby is one of the world's most respected experts on the process of regulating bacterial gene expression at the level of transcription initiation. I was awarded a **FEBS Collaborative Experimental Scholarship for Central & Eastern Europe for** this trip. While in Birmingham, I performed a series of footprinting experiments using both DNase I and a nuclease completely new at the time, FeBABE. Using the latter method enabled me to determine very precisely the position of the interaction of αCTD with the DNA of the two promoters under study, as well as to determine the orientation in which the α subunit is located in the presence of both activator proteins. In **2003,** I went again to Prof. Busby, this time for a two-week scientific consultation, during which we analysed the results obtained earlier and discussed publication concepts. At the end of this stay, I attended a two-day workshop - the **XVth RNA Polymerase Workshop** - where I gave an oral presentation (**Appendix 4, paragraph 7a [10]**). The fruits of these research internships were two publications in the journal *Nucleic Acids Research,* which were

published after I had already obtained my PhD, one in **2004** and the other in **2007** (**Appendix 4, paragraph 4b [5 and 7]**). In the first of these papers, we showed that, during activation of the p_E promoter by the CII protein, αCTD binds its determinant 265 to DNA at position -41, counting from the transcription start site, and is positioned in such a way that its determinant 261 is directed towards the σ subunit of RNAP, while the CII protein adopts an unusual location by binding on the opposite side of the double helix to RNAP. In contrast, in the second publication we showed that αCTD, when activated by the CI protein, binds in the -54 region of the p_M promoter between the O_R 1 and O_R 2 sites occupied by CI. Thus, determinant 265 of the α-subunit interacts directly with DNA, and its determinants 261 and 287 with the two dimers of the CI protein, respectively.

My dissertation, in addition to investigating the role of the α subunit in regulating transcription from CII protein-dependent promoters, included work on trying to elucidate how CII protein protection by CIII protein occurs and the mechanism of CII protein toxicity to *E. coli* cells. In the former, we showed that the CIII protein not only affects CII protein levels by blocking the action of the FtsH protease, but can also act as a specific chaperone protein, especially at high concentrations of CII. These results were reported in *Virus Genes* in **2001** (**Appendix 4, paragraph 4a [2]**). In a subsequent paper, we set out to explain why overproduction of the CII protein is strongly toxic to *E. coli* cells. Our initial hypothesis was that CII acts as a transcriptional regulator of some/any host gene(s), but upon further investigation it became apparent that excess CII drastically inhibits the replication process of bacterial DNA, probably by directly interacting, with some component of the cellular replication machinery. The results of this study were published in **2003** in *Virology* (**Appendix 4, paragraph 4a [4]**). During my PhD studies, I also participated in research on the SeqA protein, which is a negative regulator of bacterial chromosome replication initiation. We showed that this protein also participates in the development of phage λ by supporting the activation of the p_I and p_{aQ} promoters by the CII protein, whereas it does not play such a role for the p_E promoter. We showed that this action is dependent on DNA methylation and on the distribution of GATC sites, recognised by SeqA, near these promoters. These results formed part of a publication that appeared in **2003** in *Molecular Microbiology* (**Appendix 4, paragraph 4a [3]**). The results of my research from this period were presented by me or other co-authors at numerous national and international conferences, as detailed in **Appendix 4, paragraph 7a [2-10]**.

The defence of my doctoral thesis entitled "Mechanism of transcription activation by the CII protein of bacteriophage λ" took place in December **2003**. The reviewers in the doctoral thesis were Prof. Barbara Lipińska, from the Department of Biochemistry, University of Gdańsk, and Prof. Zygmunt Wasylewski, from the Department of Physical Biochemistry, Institute of Molecular Biology, Jagiellonian University.

In January **2004**, I started **a postdoctoral fellowship** in the laboratory of Dr. Finbarr Hayes at the Manchester Interdisciplinary Biocentre (MIB), University of Manchester, UK. I became the main investigator on a project entitled 'Programmed cell death in bacteria: the *yefM-yoeB* module of *Escherichia coli*' under a grant awarded to Dr. Hayes by The Wellcome Trust to understand the mechanisms regulating gene expression of the chromosomal toxin-antitoxin *yefM-yoeB* system of *E. coli*. My work was again concerned with regulatory mechanisms at the level of transcription initiation, but this time repression rather than activation of this process.

As a result of my experiments, we were able to show, among other things, that the expression of genes of the *yefM-yoeB* operon is negatively autoregulated, to show the role of the YefM and YoeB proteins in this process or to identify the precise binding sites of the YefM antitoxin to DNA. These results were published in **2007** in the journal *Nucleic Acids Research*. They form part of my scientific achievement and are therefore described in detail in **Chapter 4c**. In addition to this, I have also been involved in research on understanding the partitioning process of the pGENT plasmid from a clinical isolate of *Enterococcus faecium*. Partitioning, the active segregation of plasmid particles into progenitor cells, is an important mechanism for low-copy plasmids that would otherwise be rapidly lost from bacterial populations. In this work, we identified and analysed the centromeric sequences *cenE* of the pGENT plasmid. We found that this region has a very specific structure - it consists of three CES sites, each containing seven TATA boxes. Downstream of *cenE* is an operon encoding the *prgPO* genes, the first of which encodes the PrgP segregation protein and the second the PrgO protein, which is a homodimer that specifically binds to the CES sites. Furthermore, we showed that the *cenE* sequence has an internally curved structure that organizationally resembles yeast centromeres, which may indicate similar architectural requirements during mitotic complex assembly in yeast and in bacteria. These results were published in the journal *PNAS* in **2008** (**Appendix 4, paragraph 4b [8]**). The results of my research from my time in Manchester were presented by me or other co-authors at several conferences, as detailed in **Appendix 4, paragraph 7b [12-14]**.

After returning from my postdoctoral internship, in **2006**, I was hired as an assistant professor on a full-time research and teaching position in the Department of Molecular Biology at the University of Gdansk. I then spent almost a year on maternity and parental leave due to the birth of my first son. After returning to work, I was busy preparing teaching activities that were assigned to me as part of my salary. I also wrote a grant for my own research for the competition announced by the Ministry of Science and Higher Education, which I entitled 'Mechanism of action of the toxin-antitoxin system, Axe-Txe, derived from the pathogenic bacterium *Enterococcus faecium*'. The idea for this research arose while I was still on placement in Manchester when, in addition to experiments related to gene regulation of the chromosomal toxin-antitoxin cassette *yefM-yoeB* from *E. coli*, I conducted pilot experiments with another TA system, *axe-txe*. This module had been identified on a pRUM plasmid derived from a clinical isolate of *Enterococcus faecium* and had been pre-characterised in Dr Hayes' laboratory a few years earlier. In December **2008**, I received two pieces of news - that I was expecting my second son and that my grant proposal had been shortlisted for funding. Consequently, I did not start implementing the grant until I returned from maternity leave in **2010**. As all but one of the subsequent publications are the result of this project and form part of the scientific achievement, they are described in more detail above, in **chapter 4c**. Only the paper on the role of heat shock proteins and cobalamin in maintaining methionine synthetase activity, published in **2012** in *Acta Biochimica Polonica*, is the result of my short-lived collaboration with the group of Prof. Bogdan Banecki, from the Faculty of Biotechnology UG and GUMed (**Appendix 4, point 4b [9]**).

In **2013**, I met Dr. Luis Rios Hernandez from the University of Puerto Rico in Mayaguez (UPRM). He was invited to the University of Gdansk by the American Society for Microbiology (ASM) to conduct a workshop on research career development. Following the workshop, Dr Hernandez gave a lecture in the Department of Molecular Biology, where he

presented his research interests. These include the use of *Enterococcus* bacteria as bioindicators of faecal water pollution. In Puerto Rico, enterococci are being used to assess the purity of recreational waters in coastal areas. However, results from Dr. Hernandez's laboratory showed that these bacteria are easily adapted to live in this environment and their presence in the water samples tested does not necessarily indicate current faecal contamination. The question therefore arose as to whether it was possible to distinguish enterococci that are actually the source of faecal contamination from those that already naturally exist in coastal waters or on beaches. I suggested that enterococcal strains of different origins could be analysed for the presence of specific plasmids, which often contain antibiotic resistance determinants, virulence factors and adduct modules in the form of toxin-antitoxin systems, and see if they differed in any characteristic way. For this research, Dr. Hernandez wrote a project entitled. "The Polish connection: A tail of pheromones, adduction modules and survivability of *Enterococcus faecalis* in natural ecosystems in Puerto Rico" and was awarded a US Fulbright Foundation grant for a year's residence and research in the UG Department of Molecular Biology. From September **2015** to July **2016,** I served as supervisor and coordinator of this project. As part of the experimental work, several dozen enterococci isolates brought by Dr. Hernandez were tested. We first used pulsed-field gel electrophoresis (PFGE) to differentiate and identify bacterial strains. We gained access to the apparatus courtesy of Prof. Michał Obuchowski at the Faculty of Biotechnology UG and GUMed, Department of Molecular Bacteriology. In the meantime, we carried out a number of multiplex PCR reactions with primers specific for different types of enterococcal plasmid replication *origins*, virulence genes (*asa1*, *gelE*, *esp*, *hyl* and *cylA*) and toxin-antitoxin systems - *par*, *axe-txe*, ω-ε-ζ, *relBE* and *mazEF*. Preliminary results of our work were presented in **2016** at the *Forward Research & Innovation Summit* held in Puerto Rico (**Appendix 4 paragraph 7b [21]**). The work continued after Dr. Hernandez returned to his home unit; unfortunately, Hurricane Maria, which ravaged Puerto Rico in September **2017**, caused a power outage for weeks, which largely annihilated the strain and enzyme collection and made it impossible to complete the research. In addition, the UPRM drastically reduced research grants, so Dr. Hernandez was forced to limit his activities to academic teaching. Despite the lack of tangible benefits in terms of publications, I believe that the collaboration with Dr. Luis Hernandez has been very valuable both for me and for our Faculty. During his stay, in addition to his research, Dr. Hernandez also taught auditorium classes with biology students in a subject entitled 'Anaerobic Microbiology' and gave several lectures as part of the programme organised by the Biology Department 'Invite a scientist to school' for Tri-City high school students.

From **2014** to **2018,** I was involved in the implementation of the grant of Prof. Katarzyna Potrykus from my home unit as a consultant and expert. Due to a change in the experimental approach, the *in vitro* studies that I was supposed to personally perform were not carried out. On the other hand, since **2020**, I have been actively collaborating with Dr. Sabina Kędzierska-Mieszkowska, Prof. UG from the Department of General and Medical Biochemistry of our Faculty on a project on alternative sigma factors - ECF (extracytoplasmic function sigma factors) of the pathogenic strain *Leptospira interrogans*. I am investigating the interaction and specificity of several σ^E factors identified in this strain in autoregulation and in the regulation of expression of the *cplB* gene encoding the chaperone protein ClpB, which is also one of the

important virulence factors in Leptospira. Experimental works for one of the publications are already at the final stage of implementation.

6. **Presentation of teaching and organizational achievements as well as achievements in popularization of science or art**

(a) teaching achievements:

- Preparation and teaching (**240** hours of salary per year). For my classes (lectures, seminars, laboratories) I use my own presentations and scripts, as well as plasmid strains and vectors constructed by myself, and other teaching materials.

[1] Elements of molecular biology in environmental protection - lecture (30 hrs) and laboratories (30 hrs/group) - for 3rd year students of Environmental Protection at the Faculty of Chemistry UG - since **2008**, intermittently.

[2] Molecular biology and genetics - lecture (20 hrs) and laboratories (30 hrs/group) - for 2nd year Bioinformatics students at the Faculty of Mathematics, Physics and Computer Science UG - from **2016**.

[3] Fundamentals of biology - lecture (20 hrs) and laboratories (20 hrs/group) - for first-year students of Nuclear Safety and Radiation Protection at the Faculty of Mathematics, Physics and Computer Science, UG - from **2016**.

[4] Seminar for third-year students of Biology, Medical Biology and Experimental Biology and Genetics at the UG Faculty of Biology (30 hrs/group) - since **2008**.

[5] Speciality and diploma courses for first- and second-level students of the Molecular Biology specialisation at the UG Faculty of Biology - since **2008**.

[6] Problem based learning for third-year students at the UG Faculty of Biology (60 hrs/group) - **2013 - 2015.**

[7] Laboratories in microbiology for 2nd year students of Environmental Protection at the Faculty of Chemistry UG (30 hrs/group) - **2011**.

[8] Laboratories in molecular biology for 2nd year Biology and Medical Biology students at the UG Faculty of Biology - in different years.

[9] Half-day laboratory in Molecular Biology for third-year students of the specialisation Molecular Biology at the Faculty of Biology UG - in different years.

- Research supervision of students. I strive to involve students in my research, and as a result, three of my MSc students have become co-authors of the publications included in the scientific achievement presented here.

[1] I acted as **an assistant supervisor** in the PhD thesis of Ms Lidia Boss (dissertation entitled. "Mechanism of gene expression regulation of the toxin-antitoxin system, Axe-Txe, from the pathogenic bacterium *Enterococcus faecium"*). The thesis was defended with honours in **2015** at the Medical University of Gdansk.

[2] I have supervised **7** master's theses of Biology and Medical Biology students of the Faculty of Biology UG between **2010** and **2022**.

[3] I supervised **18** undergraduate theses of Biology and Medical Biology students of the UG Faculty of Biology between **2010** and **2022**.

[4] I supervised a Bioinformatics student from the UG Department of Mathematics, Physics and Computer Science doing an internship at the UG Department of Molecular Genetics of Bacteria in **2019**. As part of this internship, Mr Jakub Przygodzki created and tested a bioinformatics programme to search for two-codon minigenes in sequences of toxin-antitoxin systems, which was used to obtain the results described in one of my publications (**Appendix 4 point 4b [15]**).

- Thesis Reviews

I was a reviewer for **25** undergraduate and graduate theses carried out in the Departments of Molecular Biology and Molecular Genetics of Bacteria at UG between **2010** and **2022**.

- Other

The paper entitled. **"**Minigenes of the leader sequence of the transcript from the p_{axe} promoter of the *axe-txe* operon derived from the pRUM plasmid of *Enterococcus faecium*" by my MSc student Katarzyna Ogiejko was awarded in the Prof. Karol Taylor competition for the best MSc thesis done at the Faculty of Biology UG (**2017**).

(b) achievements in the field of popularisation of science

From **2008** to **2012** I took an active part in the Baltic Science Festival, an event promoting the Faculty of Biology of UG by organising various demonstrations and workshops.

(c) organisational achievements:

- Coordinator of poster sessions and undergraduate exams in the Department
- Tutor in the Department of Bacterial Molecular Genetics (since **2017**)
- Member of the UG Rector's Faculty Awards Committee for non-academic staff (term **2016-2020**)
- Member of the UG University Electoral Commission (term **2020-2024**)
- Member of the Programme Council of the Biology at the UG Faculty of Biology (from **2019**)
- Representative of assistant professors in the Council of the UG Faculty of Biology (from **2021**)

- Member of the committee preparing the self-assessment report on the Biology for the Polish Accreditation Committee (year **2022**)

7. Apart from information set out in 1-6 above, the applicant may include other information about his/her professional career, which he/she deems important.

[1] I participated in teaching conferences organised at the University of Gdansk: 1st, 2nd and 3rd National Didactic Conference "Academic didactics: tradition and modernity" (**2013**, **2014**, **2015**) and two workshops from the series Laboratory of Didactic Initiatives (academic year **2017/2018**), as well as a workshop on copyright law for the scientific community (**2019**).

[2] In February **2014**, I attended a two-day training course on Science Team Management organised by the FNP as part of the Skills project (conducted in English by Ms Susanne Marx).

[3] I participated in the 'Toxin-Antitoxin Online Seminars' organised by Laurence Van Melderen (ULB Brussels) and Pierre Genevaux (CNRS-University of Toulouse) between April **2021** and April **2022** (21 meetings in total).

[4] Awards and distinctions:

- **2022** - second level team award of the Rector of UG for leading contribution to the scientific achievement entitled "Mechanisms of interaction of bacteria with the environment".
- **2021** - second degree team award of the Rector of UG for leading contribution to the scientific achievement entitled "Molecular mechanisms of regulation of adaptive processes in bacteria".
- **2010** - Polish Genetic Society team award for the best series of genetic publications from Polish laboratories published in 2007-2009.
- **2004** - team award of the Polish Genetic Society for the best series of publications published between 2001 and 2003.
- **2003 -** Distinguished doctoral thesis.

[5] To date, I have prepared **28** reviews of articles for international scientific journals such as Toxins, Genes, Molecular Microbiology, Scientific Reports, Acta Biochimica Polonica, Frontiers in Microbiology, Microorganisms, Processes, Plasmid, FEBS Journal, FEMS Microbiology Reviews, ACS Chemical Biology, Frontiers in Cellular and Infection Microbiology.

[6] In **2014,** I was invited to give a lecture on 'Bacterial toxin-antitoxin systems' at a meeting of the Committee on Microbiology of the Polish Academy of Sciences.

[7] In **2016,** I was appointed as a reviewer for a project application submitted to the Research Foundation - Flanders (FWO), the awarding body for basic research grants in Belgium.

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(Applicant's signature)