1. Name

   Monika Katarzyna Glinkowska

2. Diplomas and academic degrees

   2003 – Ph.D. degree in biology, bestowed by the Faculty Council of the Faculty of Biology, Geography and Oceanology, University of Gdansk (currently – Faculty of Biology). Ph.D. dissertation title – "Mechanism of the p_R promoter activation and the regulation of λ plasmids DNA replication by the DnaA protein". Supervisor – Prof. dr hab. Grzegorz Węgrzyn.

   1999 – M.Sc. degree in biotechnology at the Intercollegiate Faculty of Biotechnology of Medical Academy in Gdansk (currently – Medical University of Gdansk) and the University of Gdansk.

3. Employment in scientific institutions

   2003-now Assistant Professor (adjunct) at the Faculty of Biology, University of Gdansk. Before employed at the Department of Molecular Biology and after its reorganization – at the Department of Bacterial Molecular Genetics.
   02-12.2011 – postdoctoral researcher, Jacobs University, Bremen, Germany

4. Presentation of the main scientific achievement, as defined by the Act of 14 March 2003, Article 16, Law on Academic Degrees and Title and Degrees and Title in Arts (Official Journal of Laws of 2003, No. 65, item 595, as amended).

   a) title of the scientific achievement

   The scientific achievement presented for evaluation consists of four publications containing original experimental research and one monography. They all describe mechanisms regulating DNA transactions leading to decoding the information stored in genomic DNA and its propagation in *Escherichia coli* cells. They were given a common title: „Systems biology of genomic DNA – how communication between cellular components and processes ensures efficient expression of the information stored in genomic DNA and its conveying to progeny cells".

   b) (author/authors, publication title/titles, publication date, publisher name)

      1. Szambowska A, Pierechod M, Wegrzyn G, Glinkowska M (2011) Coupling of transcription and replication machineries in lambda DNA replication initiation: evidence for direct interaction of Escherichia coli

RNA polymerase and the lambda O protein. *Nucleic Acids Res* **39**: 168–177, doi:10.1093/nar/gkq752.

$IF_{2017/2018}$ – 11.561; Ministry of Science and Higher Education (MNiSW) points $_{2013\text{-}2016}$ – 40; number of citations – 14*

Individual input into the manuscript: 40%, design of the research plan, leading role in the manuscript writing, performance of one of the experiments, cooperation in data analysis and interpretation, PI of the grant which constituted a funding source of the research, coressponding author

2. Sobetzko P, Glinkowska M, Travers A, Muskhelishvili G (2013) DNA thermodynamic stability and supercoil dynamics determine the gene expression program during the bacterial growth cycle. *Mol Biosyst* **9**: 1643–1651, doi:10.1039/c3mb25515h.

$IF_{2017/2018}$ – 2.759; MNiSW points $_{2013\text{-}2016}$ – 30; number of citations – 31

Individual input into the manuscript: 30%,co-author of the research plan and the design of the experiments, performance of 99% of the biological experiments, assistance in the manuscript writing

3. Olszewski P, Szambowska A, Barańska S, Narajczyk M, Wegrzyn G, Glinkowska M (2014) A dual promoter system regulating lambda DNA replication initiation. *Nucleic Acids Res* **42**: 4450–4462, doi:10.1093/nar/gku103.

$IF_{2017/2018}$ – 11.561; $MNiSW_{2013\text{-}2016}$ points – 40; number of citations – 5

Individual input into the manuscript: 40%, research plan design, performance of a part of the experiments, leading role in the manuscript writing, cooperation in data analysis and interpretation, PI of the grant which constituted a funding source of the research, coressponding author

4. Tymecka-Mulik J, Boss L, Maciąg-Dorszyńska M, Rodrigues JFM, Gaffke L, Wosinski A, Cech GM, Szalewska-Pałasz A, Węgrzyn G, Glinkowska M (2017) Suppression of the *Escherichia coli dnaA46* mutation by changes in the activities of the pyruvate-acetate node links DNA replication regulation to central carbon metabolism. *PLoS One* **12**: 1–24, doi:10.1371/journal.pone.0176050.

$IF_{2017/2018}$ – 2.766; $MNiSW_{2013\text{-}2016}$ points – 40; citation number – 3

Individual input into the manuscript: 30%, the main author of the research plan, performance of a part of the experiments, leading role in the manuscript writing, cooperation in data analysis and interpretation, coressponding author

5. Glinkowska M, Boss L, Węgrzyn G (2014) DNA replication control in microbial cell factories. Springer Briefs in Microbiology, Springer, ISBN-13: 978-3319105321.

$IF_{2017/2018}$ –0 (monography); $MNiSW_{2013-2016}$ points - 20;

Individual input into the manuscript: 50%, main author of the work conception, main author of the manuscript

Total Impact Factor of the presented works - 28,647; total number of MNiSW points - 170, citation number according to Web of Science - 53

c) scientific/artistic aim of the presented works and their potential application

Aim of the presented works

The aim of the works comprising the achievement presented in this application was to uncover the role of interplay between characteristic features and architecture of genomic DNA, nucleoprotein complexes responsible for DNA replication and transcription process, and cellular metabolism, in the regulation of the vital processes – gene expression and chromosome duplication.

Below I described results and conclusions of the works comprising my main scientific achievement, their scientific impact and perspectives that they created for my independent research, as well as my future scientific goals.

Introduction

Genomic DNA of one of the model bacterial organisms – *E. coli*, consists of 4,5 million base pars and around 4000 genes. Its total length exceeds the longest dimension of the rod cell more than thousand fold. Importantly, *E. coli* cells are able to change their physiology very fast in response to alterations in environmental conditions, as manifested by rearrangements of gene expression program and adjustment of the cell cycle parameters. Those facts indicate that certain mechanisms must exist which enable this seemingly simple organism to produce a particular phenotype under specific conditions, out of uncountable possible combinations of the composition and activity of its cellular components. These mechanisms must result in coordination and consistency of cellular processes, so that alteration of an environmental factor evokes reaction of the entire network of interconnected components leading to a concerted change of physiological parameters.

Reductionist approach in biology allows for identification and characterization of defined components of a living system, and even their interactions, but provides little information about the emergent properties of the system that stem from those

interconnections. Systems or holistic approaches, usually relying on simultaneous observation of many cellular components, allow to draw conclusions and formulate models of such complicated network connections and their properties, which can be further tested experimentally. Those interconnections make a living organism an autopoietic system, able to react as a unity to its environment. In my works I used both approaches to tackle following problems concerning genomic DNA transactions:

1. How is gene expression program coordinated with changing environmental conditions?
2. How is chromosomal DNA replication coordinated with cell growth and division?
3. What is the role of physical properties of DNA and genome structure in the aforementioned correlations?

Chromosomal DNA is a long and relatively rigid polymer built of nucleotides. It consists of two antiparallel strands forming the double helix. Physical properties of a given DNA fragment, like its melting temperature, intrinsic curvature or propensity to form other structures depend on base composition (G, A, T and C) and their sequence (Travers & Muskhelishvili, 2015). Those features also directly influence interactions of proteins with DNA. Their impact is the stronger, the more a protein relies on the so called indirect readout in biding DNA, meaning that it recognizes rather a particular DNA architecture than its sequence (Koudelka *et al*, 2006; Fogg *et al*, 2012; Muskhelishvili & Travers, 2016; Noy *et al*, 2016). Thermodynamic properties of a given DNA sequence determine also the propensity of the two strands to melt, in other words – the amount of energy needed to unwind them. They are of particular importance for DNA transactions which require initial strand separation like transcription and DNA replication (Muskhelishvili & Travers, 2014; Dorman & Dorman, 2016; Nigatu *et al*, 2016).

Length and rigidity of chromosomal DNA have also other consequences: in a dense environment of the cytoplasm, transcription and replication machinery introduce topological changes into DNA – additional twist of the helix. They form in the same direction as the natural winding of the helix (positive supercoils) - in front of DNA or RNA polymerases, or in the opposite direction (negative supercoils) - in their wake. Supercoiling exerts torsional tension in DNA, which may facilitate (negative supercoiling) or impede (positive supercoiling) strands separation, evoke formation of particular tertiary DNA structures or influence relative position of DNA regions due to formation of loops or plectonemes (Travers & Muskhelishvili, 2005, 2015). It means that organization of sequences within a genome and their temporary topology effectively shape genome-wide protein-DNA interactions and formation of protein complexes (Muskhelishvili & Travers, 2014, 2016). Homeostatic regulation of the global supercoiling in cells is delivered by specialized enzymes – topoisomerases. In *E. coli* it relies mainly on opposing activities of topoisomerase I, which relaxes negative supercoils and DNA gyrase, which introduces them at the expense of ATP hydrolysis. Supercoiling can thus be regarded as a form of energy storage;

considering that gyrase activity is dependent on ATP/ADP ratio, this results in tight coupling between metabolic status of the cell, DNA topology and regulation of DNA transactions – transcription and DNA replication.

Packing of the long chromosomal DNA inside the space of *E. coli* cells is ensured in part by architectural nucleoid associated proteins (NAPs), like HU, Fis, H-NS and IHF. Those proteins, by introducing bends into DNA, DNA wrapping or bridging, play a role not only in the chromosome compaction but also in regulation of transcription and DNA replication (Dorman, 2014). Chromosomal DNA of *E. coli* is in addition organized into four macrodomains: Ori (containing the *origin* of replication), Ter (comprising DNA replication *terminus*), Left and Right. The two former ones are controlled by dedicated systems consisting of specific sequences and proteins that recognize them: maoS/MaoP and matS/MatP, respectively. Moreover, between the Ori domain and domains Left and Right, there are two unstructured regions (Messerschmidt & Waldminghaus, 2014; Badrinarayanan *et al*, 2015). That organization governs chromosomal localization, segregation and the choreography of chromosomal movements in growing cells.

As already mentioned, DNA supercoiling affects transcription initiation. There are several reasons for this coupling: first of all, DNA topology influences initial DNA melting and has also an impact on the relative positioning of the key -10 and -35 promoter regions, thus effectively controlling RNA polymerase binding to these sequences. Moreover, it can enhance or reduce binding affinity of transcriptional activators and repressors, regulating their function. It is worth noting here, that the relation between DNA supercoiling and transcription is bidirectional, since – on one hand – DNA topology takes its toll on transcription initiation but on the other – RNA polymerase changes supercoiling locally while elongating an RNA chain (Muskhelishvili *et al*, 2010). Importantly, bacterial chromosome is organized in roughly 400 microdomains of approximate size 10 kb, with borders created by NAPs, among other players. Within that 10 kb distance supercoils can diffuse freely. It means that an active promoter affects the DNA architecture of neighboring regions, including other promoters and genetic elements in its cognate microdomain. Considering that the effect exerted by supercoiling on a particular DNA region depends on its sequence, we get a closed, circular system of interdependence between DNA sequence, energy stored in the form of supercoils and transcription process (Muskhelishvili *et al*, 2010). In other words, DNA encodes two types of information, one in the sequence of base pairs and another - in its three dimensional structure, whereas global transcriptional regulation may be in part an emergent property of the system, which stems from the interconnections described above (Muskhelishvili & Travers, 2013, 2014; Travers & Muskhelishvili, 2015). Although many links between physical properties of DNA and transcription process are known, the role of chromosomal architecture in global transcription regulation is poorly understood. Moreover, it remains unclear whether genome structure, i.e. positon of genes and their relative orientation harnesses transcription-coupled DNA supercoiling (TCDS) to regulate gene expression. It was also suggested in many works that TCDS may affect elements regulating DNA replication, like *origin* of replication.

One of my works, comprising the achievement presented for evaluation, focuses on the role of genome organization, physical properties of DNA sequences and changes in DNA supercoiling in global regulation of transcription program during various growth phases of *E. coli* culture (Sobetzko *et al*, 2013). In two other works, I investigated mechanisms of control of an origin of replication by transcription starting from neighboring promoters, using bacteriophage λ replicon as a model (Szambowska *et al*, 2011; Olszewski *et al*, 2014). Results presented in those publications are described in more detail below.

Another key aspect of genomic DNA biology and a classical subject of systems biology studies is the cell cycle control. The life cycle of bacterial cells consists of growth (roughly doubling of the cell mass and volume), duplication and segregation of genetic material and cell division leading to formation of two nearly identical daughter cells. Fast-growing bacteria, like *E. coli* can process the whole sequence of events in just 20 minutes, although the synthesis of entire copy of the chromosomal DNA alone takes about 40 minutes. It is possible because in those bacterial species, next round of DNA replication can start before the previous one finishes, but still only one replication initiation event per cell cycle takes place (one between subsequent cell divisions). In other words, *E. coli* cell, under conditions supporting fast growth synthesizes the chromosomes that will be inherited by her daughters and granddaughters. *E. coli* cells growing in rich media are also bigger and contain more DNA, RNA and proteins per cell than those cultivated in low nutrient media. Growing with highest rates, *E. coli* cells can contain even 16 replicating chromosome equivalents (Willis & Huang, 2017). It was shown already in the 1960s that in fast-growing bacterial species, cell size and also some other parameters, like ribosomal RNA (rRNA) content, scale linearly with growth rate, irrespective of the particular composition of the medium (Schechter *et al*, 1958). Studies carried out in the 1970s showed in addition that within the doubling time range of 20-60 min, the period necessary for the cell to synthesize the full chromosome and divide (C+D) is nearly constant and equals 60 min. Relating that data with the information about growth rate-dependent control of cell size and content lead to formulation of a replication control model stating that *E. coli* cells initiate DNA replication at constant ratio of volume to the number of *origins* (chromosome equivalents) present in the cell (Cooper & Helmstetter, 1968; Donachie, 1968). This hypothesis has been confirmed recently by monitoring growth and the cell cycle parameters of many single cells in high-throughput experiments (Wallden *et al*, 2016). Despite decades of research, we still lack answers to key questions regarding coordination of the cell cycle. Firstly, it remains unknown how the information about reaching the critical size is conveyed to the replication machinery, triggering replication initiation. Moreover, it is still poorly understood what controls cell division timing and hence – cell size and its homeostasis. A large body of evidence has accumulated over the last years indicating, that metabolic signals can be engaged in the regulation of timing of cell division and DNA replication according to the metabolic status of the cell.

In one of the works comprising the main achievement presented for evaluation I investigated an interplay between one of the important metabolic pathways (the

acetate overflow pathway) and the regulation of DNA replication. Currently, I am continuing research on interactions between *E. coli* replication proteins and metabolic enzymes or metabolites, within the frame of my two research grants. Below I described in more detail results of the work mentioned above and the aims of the two research project.

Results and their scientific impact

I took up the research topics described in preceding paragraphs in my first individual grant, financed by the Ministry of Science and Higher Education, entitled: "Transcriptional activation of the origin region – a universal mechanism to regulate initiation of DNA replication?" (44140/B/P01/2007/33). In this project we asked two main questions about the mechanisms underlying the observed impact of phage λ p_R (1) and p_O (2) promoters on the initiation of DNA replication starting from the viral *origin*.

Similarly to initiation of DNA replication from bacterial origins, copying of the λ phage genomic DNA starts from the *origin* binding and its unwinding at the AT-rich region by an initiator protein, dubbed λO. Subsequently, λP protein in association with the host DnaB helicase binds to the preformed open complex. Further remodeling of the λO-λP-DnaB complex by the host chaperone proteins DnaK, DnaJ and GrpE is necessary to release DnaB from the inhibitory interaction with λP. It had been also shown that *ori*λ requires activity of the p_R promoter for effective initiation of replication *in vivo* and in crude protein extract *in vitro*. The p_R promoter is situated upstream of *ori*λ but can be substituted for a promoter placed downstream and transcribing away from the replication starting point (Taylor & Wegrzyn, 1995). Moreover, the initiation of λ phage replicon is also influenced by activity of another promoter - p_O, localized at the beginning of the *O* gene and oriented oppositely to p_R.

In the first paper, published in ***Nucleic Acids Research*** (I am a corresponding author there) we showed that the initiator protein λO interacts directly with RNA polymerase (RNAP) of *E. coli*. The interaction between RNAP β subunit and λO is enhanced in the presence of gyrase, which introduces negative superhelical turns into DNA, thus removing positive supercoils accumulating in front of transcribing RNAP. Moreover, we obtained results suggesting that association with RNAP may influence the conformation of the initiator protein oligomer. Based on those results, we proposed a model in which the direct interaction between RNAP and λO facilitates transcriptional activation of *ori*λ provided by the p_R promoter. λO binding imposes DNA bending and enhances transcription-coupled DNA supercoiling (Leng & McMacken, 2002; Leng *et al*, 2004). This in turn affects protein-protein interactions within the λO oligomer and the complex with RNAP. We also proposed that the interaction of phage replication machinery with host RNA polymerase may play a role in spatiotemporal organization of the synthesis of viral progeny genomic DNA. Our results have also broader implications for the role of DNA supercoiling in formation of protein complexes (Szambowska *et al*, 2011).

In our next work, published in ***Nucleic Acids Research*** (I am a corresponding author of this paper) we showed that transcriptional activity of the p_R promoter is affected by the convergently oriented p_O. It results in reduction of the number of transcription events starting from p_R and traversing *ori*λ. The mechanism of that promoter crosstalk relies most likely on transcriptional interference, i.e. a roadblock formed by RNAP bound to p_O or collision of RNAPs elongating in the opposite directions. However, our results also indicated that a lack of p_O activity reduces efficiency of replication initiation from the λ *origin*, counterintuitively to its negative influence on transcription from p_R. Importantly, we showed that this interdependence between activities of both promoters and *ori*λ is true for artificial λ replicons and the phage itself. Mutation in the p_O promoter resulting in its lower affinity to RNAP, caused changes in the initiation of DNA replication suggesting weaker binding of the replication initiator to the replication origin, enhanced multimerization and instability of λ-based replicons. Considering those results, we proposed a model of regulation of DNA replication starting from *ori*λ based on transcriptional interference between p_R and p_O which controls transcription elongation through the replication origin and prevents the conflict between RNAP and the replication complex, which would otherwise lead to instability of the latter.

The results obtained during the above mentioned project have broader biological implications. They indicate how transcription and replication processes may be intertwined and how cells harness that interplay in regulatory strategies. Similar interactions are observed in eukaryotic cells regarding: coordination of transcription and replication during the S phase, influence of DNA supercoiling on formation of protein complexes, impact of RNA polymerase on DNA topology, transcriptional interference and conflicts between replication and transcription machineries.

Importantly, those results were inspirational for dr Paweł Olszewski, the first author of the second publication, to create his first individual research grant, aiming at uncovering the rules governing transcriptional interference and using them in biotechnology to regulate synthetic promoters. They were also an encouragement for me to undertake studies on the role of chromosomal DNA architecture in regulation of cellular processes. To achieve this, I started a collaboration with an expert in this field – Prof. Georgi Muskhelishvili from the Jacobs University in Bremen (Germany).

During my work as a post-doctoral researcher in the laboratory of Prof. Georgi Muskhelishvili, I was involved in a research project concerning the interdependence between genome structure (i.e. arrangement of genes and properties of their sequences), DNA topology dynamics and the regulation of gene expression during changes of *E. coli* growth phases. I participated in creating of the general research concept as well as planned and performed the biological part of experiments described below. Their results were presented in a paper published in ***Molecular BioSystems***, where I am the second author.

During exponential growth phase in rich medium, *E. coli* cells display fast accumulation of mass, accompanied by high expression of rRNA genes and the presence of many replicating chromosome equivalents. RNA polymerase associated with the σ^{70} factor, which recognizes promoter sequence, is responsible for the bulk

transcription in that phase. As the density of bacterial culture grows, nutrients become exhausted and toxic metabolites accumulate which causes activation of survival strategies. It results in cells entry into the stationary phase, characterized by inhibition of growth, DNA replication and cell division, kick-start of a gene expression program which enables survival and also – drastic reduction of ribosomal components production. It has been also shown that the entry to stationary phase is accompanied by global relaxation of chromosomal DNA and utilization of promoters recognized by RNA polymerase associated with the σ^S subunit. Importantly, the arrangement of genes along the origin-terminus axis of α- and γ-proteobacteria circular chromosomes is highly conserved. In particular, genes required for fast growth in rich media tend to be situated near the *origin*, whereas genes active in the stationary phase are localized primarily in the *terminus* region. In our research project we asked whether changes in the global gene expression program across bacterial growth phases are correlated with their arrangement along *ori-ter* axis, physical properties of genes' sequences or dependence of their promoters on the DNA supercoiling level. To tackle this problem, I performed transcriptomic analysis of mRNA isolated from *E. coli* cells growing in rich medium, within the time frame between the culture start (0 min) and the late stationary phase (430 min), with 10 min intervals between subsequent samples. During data analysis, we found out that the sequence of *E. coli* chromosomal DNA and other γ-proetobacteria displays a gradient of thermodynamic stability along the *ori-ter* axis, i.e. the energy required to unwind DNA is higher for DNA sequences in the vicinity of the replication *origin* and gradually decreases towards *terminus*.

Our results showed that upon entry to exponential growth phase, expression of genes within the Ori macrodomain and the flanking unstructured regions increases. Conversely, the entry into the stationary phase is manifested by higher expression of genes residing within the Ter macrodomain. Next, we compared gene expression timing with physical properties of their sequences and supercoiling sensitivity of their promoters, known from previous studies. We concluded that genome-wide spatiotemporal transcription program correlates with sensitivity of promoters to DNA supercoiling and thermodynamic properties of their sequences. Namely, during the exponential phase, the majority of expressed genes possess promoters activated by high negative supercoiling and sequences of high thermodynamic stability. Looking at the functional meaning of those correlations we found out that timing of transcription of those promoters correlates with high activity of genes engaged in anabolic processes and high consumption of oxygen by bacterial cells. Opposite picture emerges during entry of cells to the stationary phase. Then, mostly the genes are expressed that are connected with catabolic processes, activated by low DNA supercoiling level and having low thermodynamic stability sequences. Based on those results we proposed a model of transcriptional regulation where chromosomes function as thermodynamic machines, transforming energy into information, deciphered by transcription machinery. In this model static (physical) and dynamic (connected with changing superhelical density) properties of transcribed DNA sequences are correlated with their functional role. This ensures spatiotemporal

coordination of gene expression program during the bacterial growth cycle. In other words, it seems that bacterial genome evolved in a way that gene arrangement and physical properties of DNA are harnessed in gene expression control across various growth phases whereas dynamic DNA supercoiling couples physiological state of the cell with gene expression.

Those results have broader biological implications in understanding the role of the structural component in coding properties of DNA. Structure-function relationship is heavily studied in the case of proteins, but little is known with that respect about DNA. Uncovering those rules is important not only to understand the properties of genetic material of almost all organisms (except some viruses) but has also applicable potential in synthetic biology for construction of controlled genetic circuits and even entire synthetic chromosomes.

In addition to communication between physiological state of the cell and processes of replication and transcription operating through changes in chromosomal DNA topology, a number of publications has pointed out to direct involvement of metabolic enzymes in regulation of those processes in both prokaryotic and eukaryotic cells. In bacteria it has been shown that several enzymes affect the activity of the main division protein – FtsZ, according to concentration of important metabolites. Moreover, studies of genetic interactions suggested the possibility of a direct regulation of DNA replication by metabolic enzymes or metabolites. Such signaling could couple cell division and DNA replication with cell growth (Glinkowska *et al*, 2015).

During studies carried out after I had finished the postdoctoral fellowship, we obtained data indicating that changes in the metabolic hub connecting pyruvate, acetyl-CoA and acetate strongly affect DNA replication control in *E. coli* cells. We have also shown that alterations to cell physiology that lead to changes in protein acetylation level, redox potential, stress response evoked by increased concentration of ppGpp alarmone, all affect control mechanisms of DNA replication, connected to the DnaA protein. These results were presented in the work published in ***PLoS One***, where I am a corresponding author. Further research will shed light on the mechanisms underlying communication between those cellular parameters and the replication machinery in *E. coli* cells.

I presented current knowledge on the interplay between transcription, DNA replication, chromosome topology and metabolism as well as my own data in a monography entitled ***„DNA replication control in microbial cell factories"***. In this work we proposed also several hypotheses and further research directions in that field. Results published by other groups confirm that those topics are still of high interest.

Further research plans

Results of the works constituting my main achievement presented for evaluation, formed the basis for writing new research projects, obtaining funding and developing international collaborations.

Currently, in cooperation with my research group, we are finalizing a project which aimed at characterization of the interaction network, formed by *E. coli* replication proteins with other components of the proteome under various growth conditions. My motivation for taking up this task was again the unsolved problem in microbiology: how is the initiation of DNA replication coupled to cell growth in the cell cycle. The hypothesis underlying that project was that such coordination is an emergent property of the bacterial cell as a system and stems from many connections between the key modules as replication, metabolism, synthesis of the cell envelope and proteins. Direct interactions between replication factors and proteins engaged primarily in other cellular modules could underlie such communication.

In order to identify the protein-protein interaction network we used a method described previously by others, relying on sequential affinity chromatography. It enables isolation of bait proteins together with their interaction partners, reducing at the same time the amount of false-positive scores. Components of the complexes are subsequently identified by mass spectrometry coupled with liquid chromatography (LC-MS).

Using that method we identified direct interactions between replication factors or proteins participating in nucleotide metabolism and metabolic enzymes responsible for synthesis of the cell envelope or posttranlational protein modification. Currently we are characterizing biological function of those interactions. Each of them has a potential to become a new interesting research direction in the near future. During our work on the above mentioned project we identified also an interaction between the DiaA replication protein and a lipopolysaccharide core precursor – sedoheptulose 7-phosphate (S7P), and we proposed a model of replication control by that metabolite. S7P is not only the substrate in the first committed step to LPS synthesis but also an intermediate in the pentose phosphate pathway (PPP) producing pentose phosphate precursors for nucleotide synthesis. This metabolite could connect synthesis of the cell surface with nucleotide production and DNA replication. A manuscript presenting results on DiaA-S7P interaction is currently under revision in ***Nature Communications***. A part of the data comprising the manuscript constituted the basis for my new research grant financed by the National Science Center (NCN, OPUS No. UMO-2017/27/B/NZ2/00747). The goal of the studies planned in that project will be to uncover the mechanism of regulation of DNA replication by DiaA-S7P interaction and to characterize changes of *E. coli* metabolome along the cell cycle. I am the principal investigator (PI) of both projects mentioned above.

During realization of those grants I forged a cooperation with Prof. Torsten Waldminghaus from the Center for Synthetic Microbiology and Philipps University in Marburg (Germany). Prof. Waldminghaus is an expert in the field of chromosome biology and DNA replication and we have been using his expertise to develop our experimental skills.

I am also cooperating with dr. Manuel Banzhaf from the University of Birmingham (Great Britain) in developing a project on cell cycle regulatory mechanisms in the human pathogen - *Pseudomonas aeruginosa*. We plan to use high-throughput reversed chemical genomics for that purpose, which is a field of expertise of our

research partner. Together we aim at using the acquired knowledge to design new high-throughput tests facilitating screening for compounds inhibiting DNA replication and cell division.

Recently I have also started a collaboration with dr. Sam Meyer from the University of Lyon (France) which will enable me continuation of the studies on the relation between chromosome structure and global transcription control. Together we plan to expand the range of utilized research models and in addition to *E. coli*, perform also experiments on plant pathogen *Dickeya dadantii*, for which an impact of DNA topology on the regulation of gene expression during plant infection has already been proven. The goal of that research will be to estimate the impact of local changes in DNA topology generated by transcription-coupled DNA supercoiling and gene arrangement in the control of global gene expression programs. Dr. Sam Meyer is an expert in modeling of those processes and our cooperation will enable construction of a data-based model describing quantitatively regulation based on transcription-supercoiling coupling.

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5. Other academic achievements

In 1999 I obtained the M.Sc. degree from the Intercollegiate Faculty of Biotechnology of the University of Gdansk and the Medical University of Gdansk. My master thesis research was supervised by Prof. dr. hab. Grzegorz Węgrzyn. Its aim was to define why bacteriophage λ lytic cycle is inhibited in one of the *E. coli dnaA* mutant strains devoid of a cryptic Rac prophage. Earlier, hypothesis explaining that phenomenon suggested that a gene or a group of genes encoded by the Rac prophage substitute for DnaA function in DNA replication of the λ phage which is otherwise blocked when both the cryptic prophage and DnaA are dysfunctional. My experiments have proven that the inhibition of the λ phage lytic cycle is characteristic only for that particular strain and results from the presence of the P1prophage and inhibitory action of its restriction-modification system. Neither DnaA, nor the Rac prophage turned out to be necessary for production of the λ phage progeny. Those results were published in ***Genetics*** (Glinkowska et al., 1999).

In 1999 I also enrolled a Ph.D. program at the Faculty of Biology, Geography and Oceanology (UoG, currently Faculty of Biology). I performed the research, which constituted the basis of my doctoral thesis, under the supervision of Prof. dr. hab. Grzegorz Węgrzyn. It concerned a mechanism of transcription regulation from the λ phage p_R promoter by the host replication initiator – DnaA. That subject was interesting for me for several reasons. Firstly, the function of DnaA protein as a transcription factor had been described previously for several genes but the mechanism of regulation remained unknown both in the case of activation and repression. Secondly, control of the p_R activity by DnaA operated through binding to two sequences of weak affinity situated downstream of the transcription start site. This was one of the first regulators of that type described for bacteria, whose transcription factors typically bind upstream or within the promoter sequence. Thirdly, this topic highlighted the molecular adaptation mechanisms of the virus to its host.

Earlier studies had shown that DnaA stimulates transcription from the p_R promoter and its action is necessary for propagation of the artificial λ replicons in *E. coli* cells. In the case of the virus, a lack of DnaA activity has an impact on the viral DNA replication mode. In my work I showed that DnaA – depending on its concentration – stimulates or inhibits transcription from p_R. Cooperative binding of DnaA to two non-canonical sequences situated 5 and roughly 200 bp below transcription starting point is necessary for the regulation of p_R activity. Activation occurs by enhancing RNAP affinity for the promoter sequence and facilitating promoter clearance. These results were published in the ***Journal of Biological Chemistry*** (Glinkowska et al., 2003). A part of the research described in that paper was done in cooperation with Prof. Walter Messer from the Max Planck Institute for Molecular Genetics. Prof. Walter Messer was at that time one of the leading experts in the field of DNA replication control in *E. coli*. During three internships in his laboratory, where I spent altogether 9 months, I learnt many biochemical methods important in characterization of DNA-binding proteins.

During my Ph.D. studies I also obtained results suggesting that the DnaA46 protein variant, unable to bind ATP, which was frequently used in replication studies at that time, is unable to activate transcription from p_R and is inefficient in competition with the phage protein P for the DnaB helicase. It results in inability of plasmids containing *ori*λ to replicate in *dnaA46* strains. These results were published in Microbiology (SGM) (Glinkowska i wsp., 2001).

As a Ph.D. student I also took part in research on the mechanism of toxicity the λ phage CII protein for *E. coli* cells. Results of those studies, suggesting that CII inhibits host DNA replication were presented in the paper published in ***Virology*** (Kędzierska i wsp., 2003).

In 2004 and 2005 I received a reward for young scientists (START program) from Foundation for Polish Science (FNP).

In 2007 I obtained my first individual research grant, concerning the mechanism of transcriptional activation of *ori*λ, financed by the Ministry of Science and Higher Education. The data obtained during that project provided basis for two publications comprising the main achievement presented for evaluation and were described in more detail in the preceding paragraphs. Another result of that project was the doctoral thesis of dr. Anna Szambowska, who is a co-author in both publications. Unfortunately, polish law concerning academic degrees at that time did not provide the possibility to supervise Ph.D. thesis for PIs who did not hold habilitation degree ("supporting supervisor" nowadays).

During the same year I collaborated also on a project characterizing the role of a negative replication regulator – SeqA in the λ phage lytic cycle. In this work we showed that the effect of SeqA on the λ phage DNA replication is indirect and stems from modulation of p_R activity (Narajczyk et al., 2007).

As a PI I also collaborated with dr. hab. Marcin Łoś form the Department of Molecular Biology UoG on uncovering the role of the OxyR transcription factor in the λ prophage maintenance and induction. Results of my experiments have shown that the regulator of oxidative stress response – OxyR binds in the phage p_M promoter region. This promoter governs the synthesis of mRNA for the main repressor of the phage genes – CI. Binding of OxyR to p_M affects CI interaction with the region of overlapping, divergently oriented p_M and p_R. This way, OxyR influences both the autoregulation of p_M by CI and p_R repression, enhancing the latter and inhibiting prophage induction during the oxidative stress. These results are important considering biology of pathogenic *E. coli* strains. Their Shiga toxins, responsible for

pathogenesis, are encoded on lambdoid prophages and can be effectively produced only after prophage induction. In those strains, OxyR binding sequence at p_M is conserved. Those results were published in Archives of Microbiology (Glinkowska i wsp. 2010).

After finishing the grant concerning mechanisms of transcriptional activation of *oriλ* I worked as a post-doctoral fellow in the laboratory of Prof. Georgi Muskhelishvili at the Jacobs University Bremen (Germany). The effects of my work in his research group were presented in the publication comprising the main scientific achievement and were described in more detail above.

In 2013 my experience in research on the interplay between transcription and replication resulted in an invitation to write, together with the group of Prof. Grzegorz Węgrzyn, a review on DNA replication control. This work was published in Microbial Cell Factories (Barańska i wsp., 2013).

In 2014 and 2018 I obtained financing of two research projects concerning interrelations of *E. coli* DNA replication with other cellular processes, as described in more detail in the paragraph regarding future plans. The project that started in 2015 provided results which will be the basis of the doctoral thesis of Joanna Morcinek-Orłowska (M.Sc.). Her defense is planned at the end of 2019 or at the beginning of 2020. I will be a supervisor or supporting supervisor of her thesis, dependent on the results of this application.

Teaching and popularization of science

In the years 2003-2018 I was a supervisor of 5 M.Sc. and 5 bachelor theses. Currently I am also supervising 2 undergraduate and 2 graduate students. As a mentor I try to encourage my students to actively participate in research projects and have an individual input into project development. This approach resulted in the first authorship of my former graduate student, dr. Paweł Olszewski, in one of our works published in *Nucleic Acids Research* (Olszewski et al., 2014). One of my students, who will graduate this year, is a co-author of the manuscript currently under revision in *Nature Communications* and a review published recently in *Acta Biochimica Polonica* (Morcinek-Orłowska et al., 2019). She was also one of award winners in our group for the best poster presentation at the Central European Genome Stability and Dynamics Meeting, held in Warsaw at 2018.

During my work as a PI I supervised three Ph.D. students. The first of them, Anna Szambowska, obtained the Ph.D. degree in 2008 from the Institute of Biology (UoG) Council for the thesis entitled "The role of transcription and nucleoprotein complexes in the regulation of the λ phage and λ plasmids DNA replication". She was also a co-author of the two papers published in *Nucleic Acids Research* (Szambowska et al., 2011; Olszewski et al., 2014). Furthermore, I was a scientific advisor of Joanna Tymecka-Mulik (M.Sc.), the first author of our work published in *PLoS One* (Tymecka-Mulik et al., 2017), comprising the main achievement presented in this application. Her Ph.D. thesis is awaiting submission for evaluation. Currently I am also a supportive supervisor of Joanna Morcinek-Orłowska, who has been

employed in my grant. Her defense is expected at the end of 2019 or the beginning of 2020.

My achievements as an academic encompass also a leading role in the team establishing a new bachelor course at the Faculty of Biology (UoG) – Genetics and experimental biology (GiBE). That course belonged to the most popular students choices during the recruitment in 2018. The team was awarded be the Rector of the UoG for that project. In my work as an educator I am guided by several priority aims to be achieved with my students: conceiving and fostering the ability to identify contemporary problems in science and ask important questions, development of skills to tackle them using modern research methods. The program of the new bachelor course GiBE was created together with the Faculty of Management of the UoG in a way that reflects that concept and in addition prepares the students to implement the acquired knowledge and contribute to innovation-based economy.

During the time after obtaining the Ph.D. degree I supervised also 4 high school pupils in preparation of their experimental work for the national biology contest (Olimpiada biologiczna). Three of the pupils were awarded in the nation-wide final and one of the works gained a distinction (author: Krzysztof Sroka). Moreover, I was a mentor of the Ist High School in Gdynia pupil (Aleksander Goll) during preparation of his project for a contest for young investigators – E(x)plory. His work made it to the national final of the contest.

In 2015 and 2016 I represented the Department of Molecular Biology (UoG) at the university fair "Academia". I actively support participation of my students in the shows aiming at popularization of science like Baltic Science Festival or the Night of Biologists. The Ph.D. student I am supervising currently – Joanna Morcinek-Orłowska was a laureate of the laboratory for development of young talents in science (Kuźnia Młodych Talentów Akademii Młodych Uczonych) organized by the Polish Academy of Sciences, held in Jabłonna (Poland) at 18-21st of September 2018. She received there the 3rd price for a presentation entitled: "Tracking the messengers – interconnections between DNA replication and metabolism in bacterial cells. Together with Joanna Morcinek-Orłowska, we joined also the Spokesmen of Science (Rzecznicy Nauki) team at the UoG in 2018.

Monika Glinkowska