Author's Review of Her Research Achievements and Publications

1. First and Last Names.

Dorota Kuczyńska-Wiśnik

2. Obtained diplomas, scientific/artistic titles and degrees – with their name, place and year of their receiving and title of the doctoral dissertation.

Master of Science in biology – University of Gdańsk, Faculty of Biology, Geography and Oceanology, June 1992.

Ph.D. in biology – University of Gdańsk, Faculty of Biology, Geography and Oceanology, September 2001. Title of doctoral dissertation: "Regulation of transcription of the *ibpAibpA* operon and the role of the IbpA and IbpB proteins"

3. Information about employment at research/art centers

1993 – 2001: research-and-teaching assistant, University of Gdańsk, Faculty of Biology, Geography and Oceanology, Department of Biochemistry

2001- till now: adjunct, University of Gdańsk, Faculty of Biology, Department of Biochemistry

4. Description of the 'scientific achievement', as described in Art. 16. Section 2 of the bill published on March 14, 2003, regarding scientific degrees and titles and regarding degrees and titles in the arts (Dz. U. nr 65, poz. 595 ze zm.):

a) title of the scientific achievement

IbpA and IbpB proteins as elements of mechanism of protection of *Escherichia coli* cells against oxidative stress

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

[1.] **Kuczyńska-Wiśnik D**., Kędzierska S., Matuszewska E., Lund P., Taylor A., Lipińska B., Laskowska E. The *Escherichia coli* small heat-shock proteins IbpA and IbpB prevent the aggregation of endogenous proteins denatured *in vivo* during extreme heat shock (2002) *Microbiology* 148: 1757-1765 (IF 2002 = 2.897)

[2.] **Kuczyńska – Wiśnik D**., Żurawa-Janicka D., Narkiewicz J., Kwiatkowska J., Lipińska B., Laskowska E. *E. coli* small heat shock proteins IbpA/B enhance activity of enzymes sequestered in inclusion bodies (2004) *Acta Biochimica Polonica* 51: 925-932 (IF 2004= 1.032)

[3.] Matuszewska E., Kwiatkowska J., **Kuczyńska-Wiśnik D**., Laskowska E. *Escherichia coli* heat-shock proteins IbpA/B are involved in resistance to oxidative stress induced by copper (2008) *Microbiology* 154: 1739-1747 (IF 2008= 2.841)

[4.] Matuszewska E., Kwiatkowska J., Ratajczak E., **Kuczyńska- Wiśnik D**., Laskowska E. Role of *Escherichia coli* heat shock proteins IbpA and IbpB in protection of alcohol dehydrogenase AdhE against heat inactivation in the presence of oxygen (2009*)* *Acta Biochimica Polonica* 56: 55-61 (IF 2009= 1.262)

[5.] **Kuczyńska-Wiśnik D**, Matuszewska E, Laskowska E. *Escherichia coli* heat-shock proteins IbpA and IbpB affect biofilm formation by influencing the level of extracellular indole (2010) *Microbiology* 156:148-57 (IF 2010 = 2.957)

[6.] **Kuczyńska-Wiśnik D**., Matuszewska E, Furmanek-Blaszk B, Leszczyńska D, Grudowska A, Szczepaniak P, Laskowska E. Antibiotics promoting oxidative stress inhibit formation of *Escherichia coli* biofilm via indole signaling (2010) *Research in Microbiology* 161:847-853 (IF 2010 = 2.405)

c) Aim of the above publications and description of obtained results, including description of their possible utilization.

The aim of above publications was to expand our knowledge about the role of IbpA and IbpB the small heat shock proteins of the model bacterial organism *Escherichia coli* in protection against effects of oxidative stress.

IbpA and IbpB belong to the family of heat shock proteins called sHsp (*small Heat shock proteins*) or α-Hsp (α *Heat shock proteins*), which are molecular chaperones present in almost all prokaryotic and eukaryotic organisms. sHps share a low sequence homology (to 20%) and typical features of these proteins are: low monomeric molecular mass (15-40 kDa), the formation of large homo- and heteroligomeric complexes, the presence of a α-crystalline domain i.e. 100 amino acid residues in C-terminal region and first of all the function. Small heat shock proteins interact with substrate proteins, protecting them from irreversible denaturation and aggregation and hold them in a folding competent state. Because they do not possess ATPase activity in the process of restoring of native conformation of denatured proteins they cooperate with other Hsps. Thereby they are the essential component of network of chaperones (ClpB, Hsp70/Hsp40, Hsp60) and proteases (prokaryotic ClpXP, ClpAP, Lon) that controls folding of newly synthesized proteins and assists in the renaturation of denatured proteins or in the removing of irreversibly damaged proteins under stress conditions. sHsps constitute one of the cell's first lines of defense against stress because they are ATP-independent, have an unusually high capacity to bind substrates and are dramatically up-regulated under heat shock conditions (Laskowska & Kuczyńska-Wiśnik, 2008). Recent interest in sHsps has been propelled by the finding that they may act not only as molecular chaperones, protecting against various stress stimuli but may also play a role in many physiological processes. In mammalian cells sHsps regulate apoptosis, participate in cancerogenesis, cell development and control cytoskeletal architecture. Mutation in human shsps genes are responsible for the development of various diseases as cataract, desmin related myopathy and neuropathies and sHsps are often found as components of protein aggregates associated with protein-misfolding disorders (such as Alzheimer's, Parkinson's, Huntington's diseases) (Laskowska et al., 2010).

My research interest in the role of IbpAB proteins was a natural continuation of my PhD dissertation work, which involved regulation of expression of *ibpAibpB* genes. Results of my study indicate that mechanisms that regulate the expression of *ibpAB* genes are different from others bacterial heat shock genes and none of them have such an arrangement of regulatory elements (Kuczyńska-Wiśnik et al., 2001). These indirectly indicate an important role of IbpAB proteins in protecting of bacterial cells against stress but the knowledge was limited.

My initial work enabled me to obtain additional information about the function of IbpAB proteins in the cell. Due to the fact that lack of IbpAB proteins do not affect the cell viability, despite they are the most dramatically induced heat-shock genes in *E. coli,* I focused on phenotypic effect of Δ*ibpA/B* mutation. I showed that only under extreme heat shock at 50°C the effect of mutation can be observed: decreased viability of *E. coli* cells, increased level of aggregated proteins and slow elimination of aggregates after the recovery phase at 37°C. I observed that overproduction of IbpA and/or IbpB protein resulted in stabilization of aggregates. These *in vivo* data are in agreement with the model based on *in vitro* studies, of sHsps cooperation with ATP dependent Hsp70/DnaK chaperone system (Veigner et al., 1998). According to the current model under stress conditions IbpAB interact with non-native proteins, preventing them from irreversible aggregation, hold them in a folding competent state and facilitating their transfer to the DnaK-DnaJ-GrpE and/or GroELS chaperone system for refolding. I also demonstrated for the first time that IbpA and IbpB proteins which sharing the 48% amino acids homology exhibit different affinities to substrates. In the absence of IbpA only a small part (2%) of IbpB co-localized with the aggregated proteins whereas the majority of IbpB remained in a soluble fraction. IbpB were present in aggregates when both IbpAB proteins were produced simultaneously. However, IbpA was associated with the aggregates regardless of the presence of IbpB. The results of this research accounted for the **publication [1.]** describing *in vivo* function of IbpAB.

Prof A. Taylor research team demonstrated that IbpAB associate with endogenous protein aggregates formed following heat shock (Laskowska et al., 1996) and subsequent experiments performed by our group (Laskowska et al., 2003; Laskowska et al., 2004) revealed that IbpAB can be considered as markers for aggregates in *E. coli* cells. However, for the first time IbpA and IbpB was described as proteins associated with inclusion bodies (Allen et al., 1992). Inclusion bodies arise upon high level recombinant proteins production in *E. coli* cells when limiting availability of molecular chaperones leads to nonspecific hydrophobic interactions between polypeptides. It was known that inclusion bodies contain proteins with various conformation from entirely misfolded to properly folded proteins and process of inclusion bodies formation is dynamic - in the cell protein release from inclusion bodies and refolding occur simultaneously with protein aggregation (Carrio & Villaverde, 2002). In the subsequent publication I focused on investigation of the influence of IbpAB proteins on enzymatic activity of proteins sequestered in inclusion bodies and I found that IbpAB protect these enzymes against loss of activity. I used three expression systems for the overproduction and accumulation in the form of inclusion bodies three different proteins: Cro-β-galactosidase, β-lactamase and rat rHtrA1. I demonstrated that loss of IbpAB proteins did not affect the levels of overproduced recombinant proteins or the levels of formed inclusion bodies. However, I found about 40% higher enzymatic activity in β-lactamase and rat rHtrA1 inclusion bodies containing IbpAB than in the IbpAB – deficient aggregates. The results of these studies, presented in **publication [2.]** indicated that IbpAB bound to substrates not only protect them from irreversible aggregation and facilitate their refolding but also help them maintain the native conformation in aggregates.

Continuing studies on the role of IbpAB I turned my attention to reports indicating that these proteins participate in the protection of bacterial cells against oxidative stress. Organisms that live in

aerobic environments are exposed to reactive oxygen species (ROS), which are produced during respiration. ROS damage proteins, membrane lipids, and DNA therefore aerobic organisms have developed specific antioxidant defense mechanisms to protect the components of the cells. These defense mechanisms among others, include the antioxidant barrier formed by enzymes (superoxide dismutase, catalase, glutathione-dependent enzymes) and antioxidant small-molecules (glutathione, uric acid, vitamin C and E), which prevent the appearance of ROS and neutralize them (Nystrom, 2003). There are also reports suggesting the possible role of Hsp proteins in protection from the destructive effects of oxidation (Echave et al., 2002; Fredriksson et al., 2005; Winter et al., 2005). It was shown that overproduction of IbpAB enhances paraquat resistance in *E. coli* (Kitagawa et al., 2000) and *in vitro* IbpAB protect selected enzymes against oxidizing agents (Kitagawa et al., 2002). In our team we have undertaken the study to clarify IbpAB role in the protection of bacterial cells against oxidative stress. We demonstrated that the lack of IbpAB proteins increased sensitivity of aerobically growing bacteria to Cu^{2+} ions. The transition metal copper is an important cofactor for many enzymes but is very toxic even at very low concentrations. The mechanisms of copper cytotoxicity are caused by the fact that Cu^{2+} ions react with byproducts of aerobic metabolism: superoxide anion and hydrogen peroxide, generating highly toxic hydroxyl radicals. In the presence of oxygen, Δ*ibpAB* cells exhibit increased sensitivity to Cu^{2+} ions and accumulate elevated amounts of oxidized proteins, among which we identified the most strongly aggregating protein as alcohol dehydrogenase AdhE. We show that AdhE is protected by IbpAB against copper-mediated oxidation both *in vivo* and *in vitro*. Based on the obtained results we suggested a potential mechanism of protection, according to which the participation of IbpAB proteins in *E. coli* protection against oxidative stress involves both a direct interaction with the damaged proteins as well as preventing the formation of reactive oxygen species by binding of copper ions. Results were discussed in **publications [3**.]

In further research on the role of IbpAB proteins in protection *E. coli* cells against the effects of heat and oxidative stress we used AdhE as a model substrate. We have studied the AdhE aggregation in *E. coli* under conditions of extreme thermal shock (50°C). We have shown that under aerobic conditions IbpAB protect AdhE against thermal inactivation. We confirmed these data by the *in vitro* experiments, in which we used the purified AdhE and chaperones (**publication [4.]**). Complete reactivation of the enzyme by the DnaK / DnaJ / GrpE system occurred when IbpAB were present during the thermal inactivation of AdhE. However, it was surprising that the more rapid oxidation and inactivation of AdhE after heat shock in Δ*ibpAibpB* mutant cells was not accompanied by increased aggregation of the enzyme and the delayed removal of aggregated AdhE. Based on previously published results (**publication 1**), which showed that lack of IbpAB causes increased aggregation and delays the removal of aggregates we expected a different result. We assume therefore that IbpA and / or IbpB are not involved in the removal of irreversibly oxidized aggregated proteins destined for degradation. IbpAB protect AdhE against oxidation and inactivation and thereby maintain the enzyme in a soluble form.

In the last few years, a considerable amount of new data on the *ibpAibpB* genes expression in bacterial cells forming a biofilm has become available (Schembri et al., 2003; Ren et al., 2004;

Junker et al., 2007) although the function of IbpAB in biofilm development has not yet been established. Biofilm formation is a natural way of bacterial life. Biofilms form when bacteria adhere to solid surfaces (biotic and abiotic) on the border of the liquid phase and can be regarded as a survival strategy of bacteria in hostile environments. Biofilm development is a complex multi-step process and the transition of bacteria from planktonic (free- floating) cells to biofilm-associated (sessile) requires the coordinated expression of more than 100 genes (Harrison et al., 2007). While the knowledge about role of surface proteins in the biofilm formation is relatively broad, the role of molecular chaperones including IbpAB in *E. coli* biofilm development is still an emerging area of research. So I tried to explain this research problem. My research revealed that lack of IbpAB proteins in *E. coli* cells (strain W3110, LB broth with MOPS (pH 7, 0) inhibited biofilm formation on the air-liquid interface in PVC microtitre plates although it allowed normal planktonic growth. Moreover, I found that cells lacking IbpAB accumulated protein of approximately 50 kDa, subsequently identified as tryptophanase, *tnaA* gene product. Tryptophanase is an enzyme produced during the stationary phase of growth that converts tryptophan to indole, pyruvate and urea. Induction of tryptophanase synthesis in Δ*ibpAB* cells led to increased synthesis of indole, which is, according to literature (Wang et al., 2001; Lee et al., 2008), one of interspecies signaling molecules involved in the phenomenon of *quorum sensing* (bacterial cell-to-cell communication process) and inhibiting bacterial motility and biofilm formation. It is known (Zheng et al., 2001) that TnaA level increases during the presence of ROS; therefore I analyzed the levels of oxidized proteins and reactive oxygen species and found that cells lacking IbpAB are exposed to oxidative stress. This might results from the fact that IbpAB protect catalases against inactivation. Catalases are antioxidant enzymes involved in the removal of hydrogen peroxide from the cells. In the Δ*ibpAibpB* mutant cells activity of these enzymes was about 30% lower when compared to WT strain what might be responsible for the increased level of ROS. The results of these experiments which have indicated that IbpAB accelerate *E. coli* biofilm formation by protecting cells from oxidative stress are presented in **publication [5.].**

In the last **publication [6.]** in this series I have shown that the phenomenon of inhibition of *E.coli* biofilm formation in cells experiencing oxidative stress in an indol-dependent manner refers not only to a cells lacking IbpAB. There is growing evidence that the common mechanism of cell death initiated by some antibiotic action includes production of ROS (Kohanski et al., 2007). I demonstrated that trimethoprim, nalidixic acid, rifampicin, kanamycin and streptomycin at sublethal concentrations suppressed *E. coli* W3110 biofilm formation on the PVC surface. I confirmed that under these conditions the bacteria are exposed to endogenous oxidative stress leading to induction of *tnaA,* which catalyzes production of indole. Moreover, the lack of tryptophanase (Δ*tnaA* strain) or the presence of antioxidants such as DMSO partially restored the biofilm formation in the presence of tested antibiotics. Thus, antibiotics, which promote formation of reactive oxygen species in the bacterial cells, can inhibit formation of *E. coli* biofilm in an indol-dependent process.

In summary, major discoveries presented in the papers comprising my scientific achievement are:

- Finding that IbpAB chaperones protect *E. coli* cells against the effects of severe thermal stress by slowing protein aggregation and facilitating the removal of denatured protein aggregates at lower temperatures
- Finding that IbpAB facilitating proteins to maintain the native conformation within the aggregates
- Finding that IbpAB protect the *E. coli* cells from oxidative damage: they inhibit protein oxidation caused by either the action of Cu^{2+} as well as heat inactivation in the presence of oxygen. The protective effect of IbpAB is probably related to the prevention of inactivation of catalases
- Finding that IbpAB proteins accelerate formation of *E. coli* biofilm by inhibiting the production of indole - one of the signaling molecules that prevent cell adhesion. In the absence of IbpAB *E.coli* cells experience oxidative stress which leads to overproduction of tryptophanase and indole. I found also that the inhibition of *E. coli* biofilm development in an indol-dependent process in cells experiencing oxidative stress occurs after exposure to certain antibiotics

The results described above contribute to better understanding of the role of small heat shock proteins IbpAB in protecting cells against thermal and oxidative stress, and complement the knowledge of the molecular mechanisms of bacterial stress response.

Additional literature (except publications forming scientific achievement)

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Kitagawa M., Matsumura Y., Tsuchido T. (2000) "Small heat shock proteins, IbpA and IbpB, are involved in resistances to heat and superoxide stresses in *Escherichia coli*" *FEMS Microbiol Lett* 184(2): 165-71

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5. Description of other achievements

In 1992, I graduated with honors from the University of Gdansk, Faculty of Biology, Geography and Oceanology. Master's Thesis "Purification of RecA protein and its location in the cell membrane fractions of selected strains of *Escherichia coli* in the conditions of SOS response” I performed at the Department of Biochemistry under Prof. Alina Taylor supervision. In January 1993, I became employed as a full-time assistant in the Department of Biochemistry UG and began my academic career in a team led by Prof. Alina Taylor. At that time, together with MSc Hanna Szpilewska – my scientific supervisor, I wrote and typeset review devoted to the structure and the role of RecA protein - the subject of my Master's Thesis. Publication "Multifunctional *Escherichia coli* RecA protein - structure and functions" was published (Szpilewska & Kuczyńska-Wiśnik, 1993 - **Appendix 5, publication IID - 1**)

In my first years, I focused on several issues concerning the formation and removal of aggregates of thermally denatured proteins so-called S fraction. The S fraction is separable from the outer- and inner-membrane by centrifugation in a sucrose density gradient and it appears 15 min after a change in temperature from 30 to 45°C and lasts for only about 10 min after the temperature shift to 37°C. Also it was showed that the S fraction is stable in the *rpoH* mutant who lacks active σ^{32} , the transcriptional activator of heat-shock genes (Kucharczyk et al., 1991). We assumed that for removing the S fraction heat shock proteins function as chaperones and proteases whose genes are under the control of σ32 are responsible. Examining the process of formation and disappearance of S fraction in mutant *E. coli* strains lacking the heat shock proteases Lon, ClpP and HtrA and ATP-dependent proteins ClpA, ClpX and ClpB we found that these mutants were characterized by elevated levels of aggregated proteins and retarded removal of these aggregates. Results obtained for HtrA protease in *in vivo* studies we confirmed by the *in vitro* experiments and we demonstrated that fraction of

aggregated protein contains endogenous substrates for this protease. The results of these experiments have been published (Laskowska et al., 1996a - **Appendix 5, publication IIA - 1**). At about the same time, in the Department of Biochemistry UG two major protein components of the S fraction were identified as IbpA and IbpB proteins, discovered a few years earlier as a protein highly associated with inclusion bodies. Since 1995, returning to work after maternity I have begun research on the role and functions of these proteins in bacterial cells and successfully carry out this research today.

Initially, I conducted experiments focused on studies on the regulation of *ibpAibpB* genes expression. Both form operon controlled as well as other *E. coli* heat shock genes by σ^{32} subunit of RNA polymerase. However, my experiments showed that this is not a typical heat shock operon because IbpAB also occurred in the presence of inactive σ^{32}. Analysis of the nucleotide sequence of the *ibpAibpB* operon demonstrated in the space between *ibpA* and *ibpB* genes presence of potential additional promoter recognized by another subunit of the RNA polymerase - σ^{54} and characteristic for this type of promoter regulatory sites (the enhancer binding site and IHF binding site). I was able to demonstrate that it is an active promoter and thus transcription of the *ibpB* gene can initiate from two places: the pσ^{32} promoter common for both genes and the additional pσ^{54} present in the intercistronic region. Thus, *ibpB* is a second member of the σ^{54}-heat-shock regulon in *E. coli* besides described in 1991 (Weiner et al.) *pspA-E* (*phage shock proteins*) operon. The results of these studies have been published (Kuczyńska-Wiśnik et al., 2001 - **Appendix 5, publication IIA - 2**) and served as a basis for my Ph.D. dissertation, which I performed under Prof. Alina Taylor supervision and successfully defended in September, 2001 (title: “Regulation of transcription of the *ibpAibpB* operon and the role of the IbpA and IbpB proteins”) at the Faculty of Biology, Geography and Oceanology, UG.

After my defense, I continued my research on the function of the IbpAB proteins at the Department of Biochemistry in the following research projects (**Appendix 5, II-I 1-5**), in which I was the main investigator or the investigator. Besides research and publications described in the first part of this author review, I also participated in other projects associated with issues related to the bacterial stress responses. Among others, we started explaining the effect of the trimethoprim (TMP) action in *E. coli* cells. TMP alone or in combination with sulfonamides is one of the most frequently used antimicrobial agents which interrupt folate metabolism and thus the nucleotide and protein synthesis in bacteria. We have demonstrated for the first time that one of the effects of the TMP action in *E. coli* cells is induction of heat shock proteins - in particular IbpAB. As IbpAB associated with protein aggregates we expected that their synthesis under folate stress conditions indicates the appearance of aggregates of damaged proteins. We were able to isolate these aggregates and found most IbpAB remains associated with them. We have demonstrated that TMP caused protein aggregation and induction of main Hsps, which indicates that TMP presence leads to protein misfolding. The results of these experiments have been published (Laskowska et al., 2003 - **Appendix 5, publication IIA - 3**). In another project we also used the IbpAB as markers of aggregates this time in the study of the role of chaperone GrpE in the formation and removal of aggregated proteins. GrpE interacts with DnaK and DnaJ proteins in the process of protein folding and mutations in the *dnaK*, *dnaJ* or *grpE* gene result in similar, pleiotropic phenotypes (Liberek et al., 1991). However, while fraction of the aggregated

proteins was enlarged and stable in *dnaK* and *dnaJ* (Kędzierska et al., 1999), in *grpE* mutant cells submitted to heat shock no protein aggregation we were able to detect using previous methods such as ultracentrifugation in a sucrose density gradient or electron microscopy. Using two kinds of marker proteins: Fda (fructose-1,6-biphosphate aldolase), the previously identified aggregates component, and IbpA/B we found that in a strain lacking a functional GrpE protein aggregation occurred but aggregates are smaller than those in WT cells and cosedimented with the outer membrane fraction. In *grpE280* mutant cells we observed increase of DnaK/DnaJ and IbpAB levels after temperature elevation. In the publication containing the described results (Laskowska et al., 2004 - **Appendix 5, publication IIA - 4**) we have put forward the supposition that in strain *grpE280* formation of large aggregates is inhibited by overproduction of heat shock proteins, including IbpAB.

In the course of research on the role and properties of small heat shock proteins IbpAB I had teamed up with prof. K. Librek from the Department of Molecular and Cellular Biology, Intercollegiate Faculty of Biotechnology UG-GUM, resulted in two joint publications (Matuszewska et al., 2005 and Ratajczak et al., 2010 - **Appendix 5, publications IIA 5 and 7,** respectively). We have focused on understanding the mechanism of interaction of both proteins with each other and with the chaperones DnaK / DnaJ / GrpE. In *in vitro* experiments we showed that refolding of the thermally denatured model substrates by ATP-dependent Hsp100-Hsp70 system requires the presence of both IbpAB proteins during denaturation. In addition, we showed that *in vitro* both IbpA and IbpB proteins interact directly with each other to form mixed complexes and modulate its own activity. IbpA binds the substrates regardless of the IbpB presence but IbpB requires stimulation by IbpA. We also conducted biochemical characterization of protein IbpA (Ratajczak et al., 2010 - **publication IIA – 7, Appendix 5**), the properties of which - in contrast to IbpB (Shearstone & Baneyx, 1998), have not previously been investigated because of the difficulty in purification. We found that IbpA assembles into protofilaments which in turn form mature fibrils. These structures are unusual for small heat shock proteins, and their presence we confirmed *in vivo*. We assumed that the fibrils formed by IbpA are inactive form of the protein because they do not arise when the second small heat shock proteins - IbpB or substrate were present.

Issues connected with structure and function of small heat shock proteins with particular emphasis on their role in diseases concerns review article **Laskowska et al., 2010 (Appendix 5, publication IIA - 8**) and chapter in book (Laskowska & Kuczyńska-Wiśnik, 2008 - **Appendix 5, publication IID - 2**) while at publication Skórko-Glonek et al., 2010 **(Appendix 5, publication IID - 3**) information about the structure and function of bacterial chaperones and proteases are presented.

I also participated in a preliminary study on the heat shock response in ostracode *Candona rectangulata* naturally occurring in the tanks of Spitsbergen and characterized by rapid adaptation to changing thermal conditions (Wojtasik & Kuczyńska-Wiśnik, 2012 - **Appendix 5, publication IIA - 9**).

In addition to the mainstream of my research on the role of IbpAB proteins in protecting *E. coli* cells against stress I also participated in other projects carried out in Associate Professor Ewa Laskowska team. One of them concerned the research on bacterial cells in stationary growth phase. The consequence of depletion of nutrients which then occurs is the inhibition of bacterial growth. This is accompanied among others by reducing translation fidelity leading to the appearance in the cells

improperly folded proteins prone to aggregation (Ballesteros et al., 2001). We have shown that, depending on growth conditions and the availability of oxygen and glucose the cells accumulate complexes of Dps (*starvation-induced protein*) with chromosomal DNA or multicomponent aggregates of misfolded proteins. In the aggregates we identified proteins involved in various cellular processes such as translation, metabolism and stress response. We demonstrated that in 3-(N-morpholino) propanesulfonic acid (MOPS) -buffered cultures the level of protein aggregates and insoluble Dps was decreased. The results of these experiments were published in the publication Kwiatkowska et al., 2008 **(Appendix 5, publication IIA - 6**), and was also followed by next project, in which we analyzed the role of osmotically active compounds in the formation and disappearance of protein aggregates in the stationary growth phase. In addition, we focused on the persister cells i.e. a small fraction of bacteria that demonstrate slow metabolism and resistance to antibiotics, however, retain sensitivity to the drug after transfer to fresh medium. The presence of persistent pathogens is one of the causes of ineffective antibiotic therapy and recurrent bacterial infections, thus increasing knowledge of the mechanisms of persister's formation may help in developing new strategies to combat drug-resistant bacterial infections. The mechanisms of persister formation are largely unknown but persistence can be regarded as a symptom of bacterial aging. We found that the frequency of persisters correlated to the level of protein aggregates accumulated in *E. coli* stationary-phase cultures. At the same time we demonstrated that when MOPS or osmolyte (trehalose, betaine, glycerol or glucose) were added to the growth medium at low concentrations, proteins were prevented from aggregation and persister formation was inhibited. On the other hand, acetate or high concentrations of osmolytes enhanced protein aggregation and the generation of persisters. We have analyzed the influence of osmolytes on protein oxidation, culturability, ATP level and membrane integrity and we did not observe a link between these physiological parameters and the frequency of persisters. The results of these experiments were published this year in the publication Leszczyńska et al., 2013 (**Appendix 5, publication IIA - 10**).

Currently I continue this research in the framework of a project funded by the National Science Centre entitled "The role of protein acetylation in the formation of cells of *Escherichia coli* persisters characterized by tolerance to antibiotics". Reversible acetylation of lysine residues is post-translational modification; in *E. coli* approximately 200 proteins are acetylated (Zhang et al., 2009). The impact of this modification on the physiological processes of bacteria is poorly understood. Experiences which we have done show that the conditions that favor the acetylation i.e. the presence of glucose and acetate cause a significant increase in the level of protein aggregates and persisters in stationary cultures. We assume that acetylation of lysine residues means that proteins are prone to aggregation which leads to their reversible inactivation, metabolism inhibition and cells dormancy. My further research plans relate to clarify this hypothesis.

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