Miscellaneous

Posters

P31.1

Role of Hint1 protein in metabolism of oligo(nucleoside phosphorothioate) drugs and prodrugs

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Oligo(nucleoside phosphorothioate)s (PS-oligos) are often used as antisense therapeutics. Their hydrolysis in cellular media proceeds mainly from the 3'-end, resulting in the appearance of the nucleoside 5'-phosphorothioates ((d)NMPS). Little is known about the metabolism of these compounds *in vivo*. We suggest that the enzyme responsible for (d)NMPS catabolism could be Hint1, a phosphoramidase belonging to the histidine triad (HIT) superfamily that is present in all forms of life., because this enzyme is able to catalyze the conversion of AMPS to AMP. In our previous study, we found that (d)NMPS models were desulfured *in vitro,* in the Hint1-assisted reaction, for which the relative are as follows: GMPS>AMPS>dGMPS≥CMPS>UMPS> dAMPS >>dCMPS >TMPS, and during the reaction, H_2S was released [1]. Using RNAi technology, we have shown the lowered levels of AMPS desulfuration in the reactions that employed the cell lysates with a reduced Hint1 level [2]. In present study, we demonstrate that AMPS introduced into HeLa cells was intracellularly converted to AMP and H_2S (detected by a fluorescent assay). The level of released H_2S was relative to the concentration of AMPS used. Moreover, short PS-oligo (dApsdApsdA), electroporated into Hela cells, was metabolized to dAMP and H_2S. The results suggest the following pathway of metabolic transformations of the PS-oligo: (1) hydrolysis to (d)NMPS by cellular nucleases; (2) conversion of (d)NMPS to (d)NMP and H_2S with participation of Hint1.

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Acknowledgements:
* The studies were supported by The National Science Centre grant no DEC-2013/09/B/ST5/03612 (to 3A.K.).

P31.2

The route to protein secretion in *Bacteroidetes* phylum-revealing the role of QC and porU proteins

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In Gram negative bacteria secreted proteins have to be translocated through two cellular membranes. In *Bacteroidetes*phylum the inner membrane transfer is done by the Sec translocon, and the periplasm and outer membrane transfer by the unique type 9 secretion system (T9SS), a multiprotein complex that is now extensively studied. Deciphering the mechanism underlaying the presented protein route is of special interest because it is present in the most prominent periodontitis pathogens, namely *Porphyromonas gingivalis*and *Tannerella forsythia.* Each of the pathogen uses this system to secrete potent virulence factors.
Presented work focus on two proteins involved in secretion process. Firstly, the glutamyl cyclase (QC) protein which plays important role in the IM translocation. The QC proteins is responsible for cyclisation of cargo proteins N-terminal glutamine, which is exposed after the signal peptidase cleavage. Secondly, the porU protein which plays the final role in translocation through OM. The porU is a sortase-an enzyme that cleaves the C-terminal secretion signal (the C-terminal domain, CTD) and attaches the A-lipopolysaccharide moiety.
Here on the example of *P. gingivalis*we present data showing the influence of QC and porU deletions and mutations on bacteria survival, phenotype, expression of other T9SS components and client protein secretion and processing.

Acknowledgements:
This study was supported by the NCN funding: 2014/15/D/NZ6/02546.

P31.3

Searching for vitamin D receptor splice variants in human keratinocytes

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It is well known, that an active form of vitamin D, calcitriol (1,25(OH)2D3), modulates expression of hundreds of human genes by the activation of vitamin D nuclear receptor (VDR). However, VDR-mediated transcriptional modulation does not fully explain variety of phenotypic effects of vitamin D. Recently fast, non-genomic response to vitamin D has been described, and it seems that it does not require nuclear localization of VDR. At least three splicing variants of VDR have been described. Their cellular localization and differences in genomic or non-genomic action of vitamin D is yet unknown.
The main aim of the project is to investigate the expression level of different isoforms of vitamin D receptor simultaneously with the expression of protein involved in genomic (RXR: retinoic acid receptor) and non-genomic (PDIA3: protein disulfide isomerase family A member 3) vitamin D response in human keratinocytes.
Our preliminary data showed that at least three splicing variants of VDR receptor mRNA are expressed in human keratinocyte cells, and their expression level was changed after time-dependent incubation with 1,25(OH)2D3. In addition, it has been observed that in genes, encoding RXR and PDIA3 proteins, expression levels increase with an increase of VDR expression.

P31.4

Low molecular weight compounds which accumulate in red blood cell units increase the reactivity of blood platelets

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Bioreactive substances including cytokines, iron, lipid substances with platelet-activating factor-like activity, and other immunomodulatory substances accumulate during storage (up to 42 days) of red blood cells (RBCs) but their clinical importance remain uncertain. Supernatants from stored RBC units have been shown to induce several pro-inflammatory reactions in vitro when mixed with blood. Furthermore, RBC transfusions can cause hemostasis or bleeding disorders in recipients. The aim of the study was to investigate the effect of the supernatant from stored RBC units and its filtrate containing low molecular weight compounds (below 10 kDa) on platelet function. Adhesion of thrombin-stimulated platelets to fibrinogen and collagen (static method), platelet aggregation (turbidimetric aggregometer) and reactivity of collagen-stimulated platelets using three-color flow cytometry were measured. It was found that low molecular weight compounds which accumulate in stored RBCs increase the reactivity of blood platelets. In conclusion, supernatants from stored RBCs have pro-coagulant properties which may be the main cause of increased risk of thromboembolic events in recipients.

P31.5

Novel kinase families - unexpected biology and biochemistry in a well-known enzyme superfamily

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Within the protein kinase-like universe, despite longtime intensive research, novel kinases and kinase families still happen to be found. We employ bioinformatics tools for remote homology detection and structure prediction to identify such novel families. We will present several such examples, focusing on COTH, FAM69, and SELO.
COTH is a bacterial and fungal atypical kinase family, which we identified bioinformatically, and soon thereafter we have shown it experimentally, to be indispensable for bacterial spore coat formation by the way of phosphorylating other proteins that build up the coat (*PNAS*, 2016, 113: E3482).
FAM69 is a metazoan family of predicted kinases that we first postulated (*PLoS ONE*, 2013, 8: e66427) and then showed (*Peer J,* 2018, 6: e4599) to be involved in regulation of the secretory pathway.
The most exciting and unexpected novel kinase-like family that we have found to-date is the ubiquitous eukaryotic-bacterial SELO/YdiU family that we highlighted as unusually well-conserved family of putative enzymes (*PLoS ONE*, 2012, 7: e32138). Our recent experimental results (unpublished, under review, a joint project between groups in Dallas and Warsaw) show that SELO, although having a protein kinase-like three-dimensional structure, utilizes ATP in a manner different from all other kinases, for AMPylation (adenylylation) of protein substrates. We also present data showing that this function is conserved in SELO in evolution (bacteria, yeast, humans) and important for response to oxidative stress.
In summary, we argue that distant members of established enzyme families can turn out to perform variations of known functions or novel, unexpected functions.

P31.6

CRISPR/Cas9 and Sleeping Beauty System – novel tools for genome editing

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Nowadays, molecular biology offers great opportunities for gene manipulations in living organisms. In recent years the CRISPR/Cas9 system becomes very popular, and is wildly use for the precise genome editing in laboratories all around the world. It involves two elements: Cas9 nuclease and small guide RNA (gRNA) that directs Cas9 to desired location in the genome. There are many modifications of classic approach, one of them is all RNA approach. In this case synthetic gRNA is delivered to cells along with Cas9 mRNA through lipidmediated transfection. Such approach lowers off-target events and it is less toxic to cells in comparison to classic approach, where DNA vectors are used.
Another tool for genome editing is "Sleeping Beauty" system, based on transposition process. It is composed of transposase called Sleeping Beauty, and transposon flanked by the inverted repeats recognized by the transposase. Both elements of the system are introduced to cells in the plasmid vectors. The transposon can be any genetic sequence of interest, which by simple "cut and paste" mechanism is inserted into the genome.
Here we present our results, where we used both systems to obtain the knockout and the overexpression of MCPIP2 in U251-MG cell line.

P31.7

Disruption of the cell cycle in mucopolysaccharidoses

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Mucopolysaccharidoses (MPS) are a group of lysosomal storage diseases characterized by the progressive accumulation of glycosaminoglycans (GAGs) in lysosomes. The cause of storage is genetic defects resulting in a deficiency or complete lack of activity of specific lysosomal enzymes from the group of hydrolases. Depending on which enzyme shows reduced activity, there are 11 types and subtypes of mucopolysaccharidoses (MPS I, II, IIIA, IIIB, IIIC, IIID, IVA, IVB, VI, VII and IX). Until recently, GAG accumulation was thought to be the most important mechanism of pathogenesis in MPS, however, recent studies suggest that changes may occur in many cellular process such as the cell cycle, DNA replication, cytoskeletal function or vesicular transport.

The aim of the conducted research was to determine specific changes in the cell cycle in various types of mucopolysaccharidosis.

The research was carried out using fibroblasts from patients suffering from various types and subtypes of MPS (MPS I, II, IIIA, IIIB, IIIC, IIID, IVA, IVB, VI, VII, IX) and analogous cells from a healthy person. The cell cycle was analyzed by flow cytometry. The obtained results show changes in the cell cycle in most types of MPS compared to controls.

These results indicate that GAG storage in lysosomes is not the only disorder in mucopolysaccharidoses and may explain the current failure in the treatment of MPS focused exclusively on the reduction of GAGs levels in cells.

P31.8

QM/MM ONIOM calculations for DNA damage by solvated electrons. Optimizing a methodology for the modeling of radiotherapy-induced lesions

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Halogenated nucleobases are sensitive to the hydrated electrons formed during water radiolysis. In this study, we focus on the 8-bromoadenosine incorporated into DNA, which (after electron attachment and subsequent dissociation to bromide anion and C8-adenosyl radical) is prone to hydrogen abstraction from adjacent positions, ultimately producing cyclopurine lesions or single strand breaks.

Obtaining the free energy profiles for the lesions formation and optimizing the protocol for use in the future were the goals of the present study. ONIOM methodology in Gaussian09 was preferred as a relatively fast and straightforward method for QM/MM calculations. In this approach the system is divided into two parts: a chemically reactive region that is treated at the quantum mechanical level, and a non-reactive one that is described with molecular mechanics.

Various models and calculation protocols were tested to choose the best approach to study our target DNA system, including the size of QM and MM layers, explicit water molecules *vs.* PCM models, the employed density functional, freezing schemes, and the different starting structure geometries. Finally, results that are in reasonable agreement with those of experimental and more demanding computational methods were obtained. These also enabled the discussion on the strengths and weaknesses of the methodology, and provided guidelines to be used when modelling DNA-based systems with the ONIOM method.

Acknowledgements:
This work was supported by NCN under the Grant No. UMO-2014/14/A/ST4/00405. The calculations were carried out in WCSS, grant No. 209.

P31.9

Changes in MUPs expression patterns induced by Cbs deficiency in mice are mediated by liver Zhx2 and androgene receptor

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Major Urinary Proteins (MUPs), produced in the liver of many mammals, are excreted in the urine. In addition to participation in scent signaling trough binding of highly volatile pheromones, MUPs act as pheromones themselves or as metabolic regulators. Elevated homocysteine (hyperhomocysteinemia, HHcy) is associated with pregnancy complications in humans and female infertility in mice.To elucidate how HHcy affects sexual reproduction, we studied MUPs expression in *Cbs*$^{-/-}$ mice, a widely used model of HHcy. Real-Time RT-qPCR showed reduced Mup3 expression in *Cbs*$^{-/-}$ female mice, compared to WT animals (5-fold, $p<0.05$), in line with our previous observations of reduced urinary Mup protein levels. In contrast, male-specific Mup20 (darcin) was highly expressed in *Cbs*$^{-/-}$ females, both at the protein and mRNA levels (50.8-fold, $p<0.05$). We also found that the expression of androgen receptor (AR) was significantly reduced in *Cbs*-/- female mice vs. WT animals (20-fold, $p<0.05$). Notably, the *Timp1* gene, involved in the embryo implantation, was significantly upregulated in *Cbs*-/- females (10.16-fold, $p<0.05$), while *Zhx2* gene (regulatory element for MUPs) was downregulated both in *Cbs*-/- males (3-fold, $p<0.05$) and females (2-fold, $p<0.05$). Taken together, these findings suggest that the interaction between *Cbs* gene and MUPs, mediated by *Zhx2*, *AR*, and *Timp1* genes, is important for female fertility.

Acknowledgements:
Supported by National Science Centre 2014/15/N/NZ5/01646 grant.

P31.10

Impact of physiological concentrations of adrenaline on platelet procoagulant response, clot structure and thrombus formation under flow in human blood

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Adrenaline is a platelet activator with incompletely known mechanism of action. Conditions predisposing to the presence of elevated adrenaline levels in the body correlate with an increased risk of thromboembolism. Until now, only the effect of supraphysiological adrenaline concentrations on platelet aggregation has been studied. The potential effect of adrenaline on the platelet procoagulant response has not been specifically concerned.

In our study, we measured phosphatidylserine (PS) exposure on platelets surface and shedding of microparticles from platelets by flow cytometry, clot structure by confocal microscopy and thrombus formation under flow by flow chamber technique.

Incubation of platelets with combination of physiological (nanomolar) concentrations of adrenaline and subthreshold concentrations of collagen caused stronger PS exposure and microparticles shedding, higher procoagulant index of thrombi formed under flow, and formation of denser clots compared to control samples.

The physiological concentrations of adrenaline in combination with subthreshold concentrations of collagen induce the procoagulant response of human platelets. In addition, the changed architecture of the clots in the presence of tested adrenaline concentrations suggests the possibility of attenuated fibrinolysis. This indicates the role of adrenaline in enhancing thromboembolic tendencies through enhanced generation and retention of fibrin.

P31.11

Telomere length is not related to homocysteine and life span in cystathionine-β synthase-deficient mice and humans

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Introduction: Telomere length (TL) shortening is associated with age and elevated plasma homocysteine (HHcy), a risk factor for heart and brain diseases. Mutations in cystathionine-β synthase (*CBS*) gene cause HHcy and shorten the life span, but whether TL is involved is not known.
Aim: To examine a hypothesis that TL shortening is accelerated in *CBS*$^{-/-}$ humans and mice.
Methods: Human subjects: Czech and Polish *CBS*$^{-/-}$ patients (n=21) and controls (n=27), age 0.1–57 years. Mice: *Tg-I278T Cbs*$^{-/-}$ and *Cbs*$^{+/-}$ siblings, age 36–408 days, n=79. Relative TL was quantified using DNA from whole blood, mouse liver, and brain in qPCR assays (Cawthon R, *NAR* 2009). Levels of telomerase (Tert) and senescence markers (IL-1B, MCP1, IL-6, p21, PAI-1, Kl) mRNA were quantified using total mouse liver and brain RNA in RT-qPCR assays, and normalized to b-actin and Gapdh mRNAs.
Results: Leukocyte TL was inversely correlated with age but CBS deficiency did not accelerate age-dependent TL shortening in humans or mice. TL was not associated with HHcy in mice (leukocytes, liver, and brain). Tert was reduced in *Cbs*$^{-/-}$ mice. MCP1 and p21 were elevated, IL-1B and IL-6 reduced in the *Cbs*$^{-/-}$ mouse liver but not in brain. PAI-1 and Kl were unaffected.
Discussion: Cbs deficiency, HHcy, and associated premature death are correlated with senescence markers in the liver, but not with leukocyte TL in mice. CBS deficiency does not affect TL in humans.

Acknowledgements:
Supported by NCN grants 2015/17/N/NZ3/03626 and 2016/23/B/NZ5/00573.

P31.12

In search for pectinolytic bacteria in Polish lakes

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The *Pectobacteriaceae* family encloses *Pectobacterium* and *Dickeya* genera into which important pectinolytic plant pathogens responsible for blackleg and soft rot diseases of crops and vegetables are classified. The economic losses solely on potato reach 250 million euro annually. Our aim was to search for *Dickeya* and *Pectobacterium* spp. strains at different depths of 9 Pomeranian lakes in order to estimate whether these reservoirs are important sources of disease-causing agents. During 12 months of study, water samples have been collected monthly from all lakes by a qualified scuba-diver. To species identification was achieved by multiplex PCR and species specific PCRs resulting in detection of 10 isolates of *Dickeya* spp. and 5 isolates of *Pectobacterium* spp. Further studies lead to identification of *Pectobacterium carotovorum* subsp. *brasiliensis* (Pcbr) in water reservoirs. Notably, this species was not reported within the Polish waterways before. The identification of Pcbr isolates was confirmed after analysis of the sequences of *dnaX* and *recA* genes. Characterisation was based on phenotypic features such as pectinase, cellulose, protease activities, motility, siderophore and virulence on potato. Interestingly, all the obtained pectinolytic bacteria have been collected from 0 m depth nearby the shore. Therefore, usage of irrigation water originating from depths instead of the surface water, might contribute to limitation of the spread of soft rot *Pectobacteriaceae*.

P31.13

Thymoquinone effectiveness and mechanism of antibacterial action against *Staphylococcus aureus*

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Staphylococcus aureus can be both a commensal organism and a pathogen. It is carried by approximately 30% of human population [1]. *S. aureus* is one of leading causes of bacteremia, infective endocarditis and osteoarticular, skin and soft tissue, pleuropulmonary and device-related infections [2] Wide spectrum of possible pathogenesis caused by *Staphylococcus aureus*, its pervasiveness and increasing resistance to antibiotics creates the urge to search for new therapeutics effective against it [3].
Thymoquinone is a part *Nigella sativa* seeds essential oil and was reported to have anti-oxidative, anti-inflammatory, immunomodulatory and anti-microbial activity. In addition to thymoquinone, significant amounts of thymol and p-cymene are present in black cumin essential oil.
In this work oils obtained using supercritical carbon dioxide extraction method from *Nigella sativa* seeds and standards of thymoquinone and thymol – primary biologically active substituents of the oil – were used against various strains of *Staphylococcus aureus*, including methicillin-resistant ones. Bacteriostatic and bactericidal activity as well as inhibition of biofilm formation were analysed using broth dilution methods. Fluorescent probes and direct methods were facilitated to analyse influence of used substances on cell membranes and intracellular generation of reactive oxygen species.

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P31.14

Is RAB GTPase homolog E1B (RABE1b) involved in response to cadmium stress in *Arabidopsis thaliana* seedlings by cGMP synthesis?

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Cyclic nucleotides have been shown to play important signaling roles in plants including defence against biotic and abiotic stresses. During our studies on *Arabidopsis* responce to cadmium stress (50 mM), we found that with GTP-agarose binds protein with guanylate cyclase (GC) activity. After SDS-PAGE, the 52 kDa protein was subjected on Orbitrap spectrometer and MASCOT analyses and it showed that it is RAB GTPase homolog E1B (RABE1b, At4g20360). Next we found modified GC motif: **S**F**T**-VRAARGKFER**K**, which has 12 out of 14 amino acids typical for that motif [1].
In our studies on cadmium stress we showed fraction eluted from GTP-agarose that had GC activity and during experiment conducted up to 24 h the highest activity was observed at 10 h (about 2.5 pmol cGMP×mg^{-1} proteins×min^{-1}) and it was about 30-fold higher than in controls. Analysis of *RABE1b* gene expression revealed its induction by cadmium that was after 30 min 38-fold higher than in controls. We also tested gene expression of two other proteins with GC activity and GC motif: *AtGC1* [2] and *AtBRI1* [3] and it was about 2- after 3 h and 2.5-fold after 1 h higher than in control seedlings.
Our studies indicate that RABE1b is involved in response to cadmium stress. By study overexpression of RABE1b *in vitro* we expect to obtain an answer to the question if this protein is a "new" plant guanylate cyclase.

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