
Session IX: Spotlights on Polish life sciences

Lectures

L9.1

What is the contribution of replication initiation protein to strand-specific replisome assembly?

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Keywords: DNA replication; Rep protein; replisome; ss-DNA

Research on DNA replication has not revealed the exact mechanism for replication complex *de novo* assembly nor how the directionality of replication is determined. By using broad-host-range plasmid system we demonstrated evidence for direct involvement of a replication initiation protein in the process of polymerase recruitment. Through interactions with 13-mer sequences on one strand of initially unwound DNA [1] and with the subunits of DNA polymerase [2], the initiation protein facilitates strand specific replisome assembly at the plasmid replication origin. This step determines the direction of DNA replication. Interaction of replication initiator with β -subunit of DNA polymerase has been identified as an essential contribution of the plasmid replication initiation protein to the process of the replisome assembly. We propose models of the plasmid replication initiation protein and its complex formed with single stranded DNA of replication origin. Our study provides new insights into the understanding of replication initiators activities.

[1] Wegrzyn K, Fuentes-Perez ME, Bury K, Rajewska M, Moreno-Herrero F, Konieczny I. (2014) *Nucleic Acids Res.* 42(12):7807-18
[2] Wawrzycka A, Gross M, Wasznik A, Konieczny I. (2015) *Proc Natl Acad Sci USA.* 112(31):E4188-96

L9.2

Chatty mitochondria: Keeping the balance in cellular protein homeostasis

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Keywords: mitochondria; proteins; organellar stress
Mitochondria need more than one thousand cellular proteins to fulfill various functions. Yet, the majority of mitochondrial proteins are synthesized outside mitochondria in the cytosol and thus must be efficiently sorted into mitochondria with the help of translocation machineries. Dysfunctional mitochondrial protein import causes mitochondrial malfunctions, but also accumulation of precursor proteins in the cytosol.

We undertook the proteomic approach to understand the consequences of defects in the mitochondrial protein sorting. Our effort resulted in a comprehensive and quantitative characterization of changes in the proteome of cells with a defect in the import of proteins into mitochondria. Changes in several biochemical paths were identified, which can be involved in maintaining homeostasis and survival of the cells exposed to a mitochondrial import defect. Two main arms of the response, which protect against mitochondrial protein import defects, include the inhibition of cytosolic translation and activation of the major protein degradation machinery, the proteasome. The stimulation of the proteasome was driven by its more efficient assembly as a direct response to the amount of mistargeted proteins. The mechanism is beneficial for cells. Interestingly, the synthesis of cellular proteins is regulated by signals, which come directly from the dysfunctional mitochondria. This findings uncover a crosstalk between the state of mitochondria and regulatory mechanisms responsible for maintaining the cellular protein homeostasis.

Posters

P9.1

Aminophosphinic urease inhibitors as permeabilizers

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Keywords: urease inhibitors; *Proteus*; permeability

The use of pharmaceuticals affecting the integrity of the outer membrane of Gram-negative bacteria is a known way of increasing pathogens susceptibility to hydrophobic antimicrobials. In the presented research, the ability of 8 aminophosphinic compounds to increase membrane permeability was evaluated. The compounds described are already known as inhibitors of *Proteus mirabilis* urease. The assumption of their possible membrane permeabilizing effect was based on uncommon results obtained while testing for bacteria viability using MTT metabolic efficiency assay and fluorescence staining. The increasing susceptibility of *P. mirabilis* to chosen antibiotics: erythromycin, vancomycin and novobiocin was evaluated using the standardized broth dilution method and this allowed us to identify three compounds with the most significant permeabilizing activity, namely bis(aminomethyl)phosphinic, bis(N-methylaminomethyl)phosphinic and aminomethyl(N-n-heptylamino-methyl)phosphinic acid. The examined compounds alone did not exert any antibacterial effect in Mueller-Hinton medium. Incubation with 0.5 mM concentration of these compounds produced a decrease of the MIC values of erythromycin by 21%, 23% and 16% respectively. The *Proteus* strain treated with bis(aminomethyl)phosphinic acid was also more susceptible to vancomycin with an MIC value at the high but measurable level of 850 μ M. Both of these antibiotics are considered to be inactive against *Proteus* strains. A higher permeabilizing activity of the examined compounds was observed after coincubation with novobiocin – an antibiotic effective against *P. mirabilis* with MIC₅₀ of 2.4 μ g/mL. Susceptibility was increased by 36%, 68% and 73%, respectively. In conclusion, the presented characteristics is a promising factor, considering the main antiureolytic activity of the compounds presented.

P9.2

Under hyperglycemia, the increased activity of mitochondrial uncoupling protein 2 improves stress resistance in human endothelial cells EA.hy926

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Keywords: endothelium; mitochondria; uncoupling protein

We describe the functional characteristics and an antioxidative role of uncoupling protein 2 (UCP2) in endothelial EA.hy926 cells and isolated mitochondria. We examine the influence of high-glucose exposure on UCP2 activity and ROS production in endothelial cells. The UCP2 activity was significantly higher in mitochondria isolated from high glucose-treated cells. In high-glucose endothelial cells, the increased activity of UCP2 led to improved stress resistance and protection against acute oxidative stress. Our results indicate that endothelial UCP2 may function as a sensor and negative regulator of mitochondrial ROS production in response to high glucose concentration.

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P9.3

The response of endothelial mitochondria to elevated level of free fatty acids

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Keywords: mitochondria; endothelial cells; oxidative metabolism

INTRODUCTION: Endothelial cells have permanent contact with blood and transported compounds, including free fatty acids (FFAs). A chronic elevation of circulating FFAs is observed in obesity and diabetes type 2. The goal of this study was to assess the influence of exposure to elevated FFA level on the aerobic metabolism of endothelial cells.

MATERIALS AND METHODS: A permanent human endothelial cell line EA.hy926 was chronically (for 6 days) exposed to elevated level of palmitic acid (tested range 100 – 150 μ M). We examined changes in respiratory functions in endothelial cells measuring: oxygen uptake by cells with different respiratory substrates; total and mitochondrial reactive oxygen species (ROS) formation, protein expression level of marker proteins of glycolysis, anaerobic metabolism, β -oxidation, tricarboxylic acid cycle and mitochondrial respiratory chain.

DISCUSSION: Our data demonstrated that the elevated level of FFAs significantly affects endothelial oxidative metabolism, ROS formation and cell viability. Thus, it might contribute to endothelial dysfunction, that represents a key early step in the development of e.g. atherosclerosis. Emerging experimental evidence, including our results, suggests an important role for endothelial mitochondria in the pathomechanisms of many cardiovascular diseases.

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P9.4

Role of Arginase 1 in parasite-induced immunosuppression

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Keywords: Arginase 1; intestinal nematode; immunosuppression

Arginase 1, an enzyme converting L-arginine to ornithine and urea, has various functions in mammals. It is particularly important in immunity as it participates in immunosuppression. Intestinal nematodes are pathogens that strongly inhibit immune response via the induction of AAMf, Treg, TGF β , IL-10 production, etc. The aim of the study was to determine the role of Arginase 1 during parasite-induced immunosuppression.

Methods: Male BALB/c mice obtained 1% L-arginine in drinking water or the Arginase 1inhibitor BEC (1 μ g/g body weight daily) via oral gavage for 26 days. On the second day of treatment, the mice were infected orally with 200 L3 larvae of the *Heligmosomoides polygyrus* nematode. Untreated mice served as controls. Twenty-five days after infection, the number of adult worms in the small intestines was counted. Additionally, Gr-1+ cells were isolated from the peritoneal lavages using magnetic beads and cultured in a ratio of 1:40 for 5 days with TCR-stimulated, CFSE-stained CD4+ splenocytes from uninfected mice. The proliferation of lymphocytes was measured using flow cytometry.

Results: Mice that were treated with L-arginine and BEC exhibited reduced levels of infection, and this effect was particularly strong for BEC. We connected this finding with the reduced suppressive activity of peritoneal Gr1+ cells; cells from treated animals resulted in weaker inhibition of CD4+ proliferation. These results suggest that Arginase 1 and Gr1+ cells are one more type of immunosuppression mechanism used by *H. polygyrus* to remain within the host.

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P9.5

Isolation, screening and initial characterization of biosurfactant-producing microbes from water and foam samples

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Keywords: biosurfactants; screening; lipopeptides; rhamnolipids

Biosurfactants are surface active molecules of microbial origin. Biosurfactants exhibit similar properties to synthetic detergents but are environmentally friendly and have compelling antimicrobial, antiadhesive or anticancer activities [1]. Biosurfactant activity depends on molecular structure, and therefore screening for new biosurfactants can potentially identify molecules with remarkable activities [2].

We report isolation and screening for biosurfactant-producing microbes from water and foam samples. Three-stage screening strategy selected 78 possible biosurfactant producers from 221 isolated unique strains and then limited this number to 48 efficient biosurfactant producers. Thirty-seven of these bacteria were isolated from foam and only 11 from water samples indicating enrichment of foam with biosurfactant producers. Almost 90% of isolated biosurfactant producers were Pseudomonads. PCR detection of nonribosomal peptide synthetases (NRPS) and rhamnolipid (*rhl*) gene clusters allowed assignment of 40 strains as lipopeptide and 15 as rhamnolipid producers. Culture conditions for biosurfactant production by 8 strains were optimized. Raw biosurfactants were isolated and initially analyzed using TLC, HPLC and UPLC-MS. Extracts were further fractionated by semi-preparative HPLC and individual fractions were tested for surface activity. Our results indicate lipopeptides are produced by six strains and rhamnolipids are produced by two strains.

[1] Janek T, Krasowska A, Radwańska A, Łukaszewicz M (2013) Lipopeptide biosurfactant pseudofactin II induced apoptosis of melanoma A 375 cells by specific interaction with the plasma membrane. *PLoS One* 8 (3) e57991.

[2] Biniarz P, Łukaszewicz M, Janek T (2016) Screening concepts, characterization and structural analysis of microbial-derived bioactive lipopeptides: a review. *Crit Rev Biotechnol*.

P9.6

The influence of the overexpression of *GUT1*, *SCT1* and *DGA1* genes on lipids production from glycerol by *Yarrowia lipolytica*

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Keywords: metabolic engineering; lipids production; oleaginous yeast; glycerol

In the modern world, fossil fuels are the basis of a large branch of industry, like factory farming or heavy transport. Fossil fuels are likely to soon be exhausted and plant energy resources will compete with food production for farmland. The oleaginous yeast *Yarrowia lipolytica* is one of the most promising biological platforms for industrially valuable oil production. Lipid synthesis in cells depends on the activity of various enzymes. In this study, we tested the impact of overexpression of 3 native genes on lipid production from glycerol. We used glycerol as a substrate because of the large global quantities produced and its low value. It is essential to find new solutions to convert this substrate into a value-added product. To enhance the production of lipids from glycerol, we overexpressed the *GUT1* (*YALI0F00484g*) gene coding glycerol kinase, which enables the conversion of glycerol to glycerol-3-phosphate. Subsequently, to direct carbon flow into lipid production, we overexpressed the *SCT1* (*YALI0C00209g*) gene encoding glycerol-3-phosphate - acyltransferase. Final acylation occurs by diacylglycerol acyltransferase, which is encoded by the *DGA1* (*YALI0E32769g*) gene. Overexpression of *DGA1* yielded a 2.5-fold increase in lipid production over the control and a 1.8-fold increase in the lipid content of dry cell weight. Next, we combined all 3 genes for co-overexpression, and the resulting strain *GUT1+SCT1+DGA1* yielded a 2.8-fold increase in lipid production. This work demonstrates that biosynthesis pathways are subject to multi-level influence, but the overexpression of the *DGA1* gene is vital for lipid production. Effective metabolic engineering may create biological platform for efficient biodiesel production by microorganisms.

P9.7

Modified ADM1 model for simulation of multi-chamber anaerobic digester

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Keywords: anaerobic digestion; ADM1

Anaerobic digestion model no.1 (ADM1) is an accepted model for simulation of anaerobic digestion. However the basic version of ADM1 omits several biological processes [1] that may be essential for simulation of biogas production, such as formation of lactic acid. This process is crucial for digestion of dairy byproducts in high-rate systems. An example of such a system is tubular bioreactor (TB), which consists of six 3 l compartments connected in line. The hydraulic retention time for individual compartments is 3 days. During operation of this bioreactor set, the fermentation conditions in individual compartments are differentiated. In the first two compartments, lactic acid accumulates, resulting in a sharp pH decrease. In subsequent tanks, the acid is consumed and the pH increases to 7 in the last compartment. The amount and composition of biogas produced in individual compartments differed between each other. Molecular hydrogen was detected in the gas formed in compartments 1, 2 and 3. Acid composition, hydrogen production and pH in individual compartments of TB were not reproduced by the original ADM1. The introduction of lactic acid fermentation into the ADM1 was sufficient to simulate fermentation conditions in TB.

[1] Jabłoński S, Łukaszewicz M (2014) Mathematical modelling of methanogenic reactor start-up: Importance of volatile fatty acids degrading population. *BioresTechnol* 174: 74-80.

P9.8

The neuroprotective effects of *Anemarrhena asphodeloides* root extract – an *in vitro* study

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Keywords: neuroprotective; *Anemarrhena asphodeloides*; Alzheimer disease; Parkinson disease; PC12 cell line
Neurodegenerative diseases are among the most serious diseases of modern societies. The most common diseases in this group are Alzheimer's and Parkinson's disease. Plant extracts are a promising class of therapeutics for the treatment of neurodegenerative diseases. The extract of *Anemarrhena asphodeloides* has been used in Asian traditional medicine for many years and has well-known antidiabetic, antioxidant and antitumor properties. Studies have also confirmed its neuroprotective bioactivity, as well as the cytotoxicity of some saponins isolated from *A. asphodeloides* root extract.

Here we aimed to evaluate the neuroprotective properties of *A. asphodeloides* root extract in PC12 rat pheochromocytoma cells. We used root extract of *A. asphodeloides* in concentrations from 5 to 100 µg/mL and MTT viability assays were performed at 8, 16, 24, 48 and 72 h after treatment. The MTT assay is a commonly used method to evaluate cytotoxicity by measuring the oxido-reduction mitochondrial activity. To evaluate the neuroprotective properties of *A. asphodeloides* root extract, we have provoked cytotoxicity using 3-nitropropionic acid (3-NP) in concentrations from 2.5 to 15 mM.

We found an increase in cell survival after treatment with the root extract, suggesting a cell regenerative effect. Based on these findings, we propose that *A. asphodeloides* has neuroprotective properties in rat pheochromocytoma cells *in vitro*.

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P9.9

The influence of chemical structure of novel surfactants on the antibacterial activity and their interactions with DNA

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Keywords: surfactants; biofilm; adhesion

The studies were aimed to contribute to the elucidation of the relationships between structure of the novel cationic surfactants - N,N-bis[3,3'-(dimethylamino)propyl]alkylamide dihydrochlorides and N,N-bis[3,3'-(trimethylammonio)propyl]alkylamide dibromides (alkyl: *n*-C₉H₁₉, *n*-C₁₁H₂₃, *n*-C₁₃H₂₇, *n*-C₁₅H₃₁) and their biological activity. The antibacterial activity of the compounds as well as their influence on the adhesion and biofilm formation of two bacterial strains - *Pseudomonas aeruginosa* PAO1 and *Staphylococcus epidermidis* ATCC 35984 was investigated. Tested surfactants exhibited antibacterial activity. All of the tested surfactants showed the ability to condense DNA.

P9.10

The Lewis acid – catalyzed rearrangement of vinyl ethers and alkoxydienes

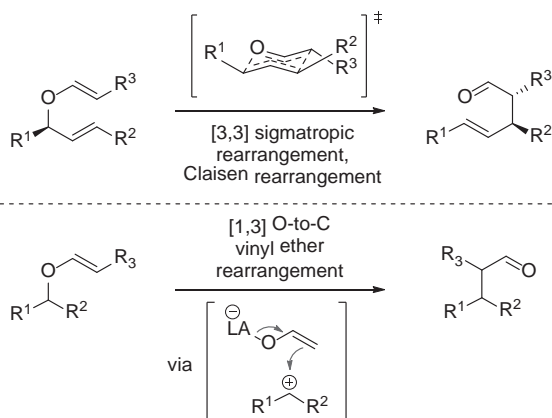
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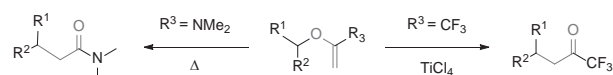
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Keywords: catalyzed rearrangement; vinyl ethers; alkoxydienes

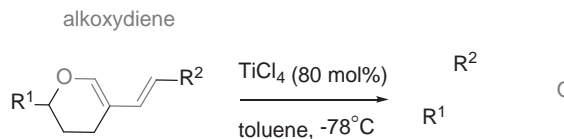
Vinyl ethers undergo two particular transformations. One of them is the [3,3] sigmatropic rearrangement of allyl vinyl and the allyl aryl ethers, known as the Claisen rearrangement, which leads to γ,δ -unsaturated carbonyl compounds. On the other hand, a less well-known transformation of vinyl ethers is the Lewis acid-catalyzed [1,3] rearrangement (Nasveschuk *et al.*, 2005; Nasveschuk *et al.*, 2008).



We have discovered that highly functionalized vinyl ethers undergo smooth oxygen-to-carbon rearrangement in the optimized conditions. Of most interest remains the possibility to obtain such valuable compound as trifluoromethyl ketones or amides.



Furthermore, we have shown that alkoxydienes undergo smooth rearrangement in a vinylogous manner with a catalytic amount of TiCl₄, which leads to highly substituted cyclohexenes.



References:

- Nasveschuk ChG, Rovis T (2008) The [1,3] O-to-C rearrangement: opportunities for stereoselective synthesis. *Org. Biomol. Chem.* 6: 240-254. doi: 10.1039/b714881j
 Nasveschuk ChG, Rovis T (2005) Regioselective Lewis Acid-Mediated [1,3] Rearrangement of Allylvinyl Ethers. *Org. Lett.* 7: 2173-2176. doi: 10.1021/ol0505151

P9.11

Generation of various human SLC35A3 knock-out cell lines using the CRISPR-Cas9 approach

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Keywords: UDP-N-acetylglucosamine transporter, CRISPR-Cas9, knock-out

The CRISPR-Cas9 technology is derived from a type II bacterial immune system and represents a new method of targeted genome editing technology, which can be applied to almost all living organisms. This technique employs the nuclease Cas9, the crRNA array encoding gene-specific guide RNAs, and a required auxiliary trans-activating crRNA.

In our laboratory, the CRISPR-Cas9 system was used to knock-out the SLC35A3 gene in several human cell lines. The product of this gene is considered the main mammalian UDP-N-acetylglucosamine transporter. Each step of the SLC35A3 knock-out process required optimization. It was challenging, because there are significant differences among the various cells lines. We tested a wide spectrum of conditions (e.g., concentration of selection antibiotic, plates with different surfaces, culturing conditions, etc.).

After transfection, cells were selected in complete media containing puromycin. We were able to distinguish non-transfected cells from the potential SLC35A3 knock-out cells using fluorescence microscopy, because the plasmids employed in the study encoded both RFP and GFP. After selection, SLC35A3 expression was investigated by PCR.

We knocked-out the SLC35A3 gene in three cell lines: HEK293T, HeLa, and HepG2. The resulting clones had different morphology when compared to the wild type cells. Their growth rate was decreased and they adhered poorly to plates. For the HeLa cells, we found that the generated clones cannot be stored in liquid nitrogen, because they do not survive the thawing procedure.