Session 14. Bioenergetics

Lectures

L14.1

Mitofusin deficiency affects energy metabolism, mitochondrial biogenesis and mtDNA content in MEF cells

Maria Kawalec[1], Anna Boratyńska-Jasińska[1], Małgorzata Beręsewicz[1], Dorota Dymkowska[2], Dagmara Kabzińska[3], Barbara Zabłocka[1], Krzysztof Zabłocki[2]

[1]Molecular Biology Unit and [3]Neuromuscular Unit, Mossakowski Medical Research Centre, PAS, Warsaw, Poland; [2]Department of Biochemistry, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland
e-mail: Krzysztof Zabłocki <k.zablocki@nencki.gov.pl>

Mitofusin 2 (Mfn2), mitochondrial outer membrane protein which is involved in its rearrangement, was first described in pathology of hypertension and diabetes, but nowadays much attention is paid to its functions in Charcot-Marie-Tooth type 2A neuromyopathy (CMT2A). Here, effects of Mfn2 deficiency on cell metabolism in the presence or absence of Mfn1 were investigated. Experiments were performed on three lines of mouse embryonic fibroblast (MEFwt, $MEF^{Mfn2-/-}$ and $MEF^{Mfn1-/-Mfn2-/-}$). Substantial reduction in the maximal cell respiration rate in KO-cells, which was accompanied by significant decrease in complex I and IV subunits content in double knockouts. Conversely, $MEF^{Mfn2-/-}$ cells exhibited significantly increased respiration rate in comparison to found in MEFwt cells. These effects were correlated with differences in mitochondrial markers (TOM20 and HSP60) as well as TFAM protein levels. Despite that, total ATP content was the same in all cell lines tested. Interestingly, an inhibition of mitochondrial ATP-ase with oligomycin in the absence of glucose in the medium did not significantly reduced ATP content in $MEF^{Mfn1-/-Mfn2-/-}$ suggesting more pronounced participation of glycolysis in ATP synthesis in these cells that found in the wild type ones. It correlates with substantially higher amount of glycogen stored in KO cells in comparison to wild type MEF. Therefore, it seems that mitofusins deficiency causes a shift in metabolism from oxidative to anaerobic. Additionally, the reduced level of mtDNA in $MEF^{Mfn1-/-/Mfn2-/-}$ cells is in a good correlation with lowered TFAM. It may be an important feature of CMT2A pathology and the contribution of Mfn1 shouldn't be omitted especially that CMT2A-causing mutations have distinct organ-specific effects. Overexpression of wild type mitofusin 2 gene in $MEF^{Mfn1-/-/Mfn2-/-}$ restores the normal mtDNA content while introduction of CMT2A-related mutations in *MFN2* does it in significantly lower rate.

Acknowledgements

The project is supported by NSC grant NN402474640.

L14.2

Fluorescence microscopy of single molecules

Rafal Luchowski, Monika Zubik, Wojciech Grudzinski, Joanna Bednarska, Ewa Janik, Wieslaw I. Gruszecki

Institute of Physics, Department of Biophysics, Maria Curie-Sklodowska University in Lublin, Lublin, Poland
e-mail: Rafał Luchowski <rafal.luchowski@umcs.pl>

Fluorescence technology is being widely used in biological research and medical/clinical diagnosis. In last decade, fluorescence detection has been developed to selectively address individual molecules. Single molecule detection (SMD) provides several crucial advantages over more conventional bulk methods, for biological measurements. By examining a separate systems, it is possible to avoid averaging effect or have a deeper insight into the individual properties of the measured sample.

Over the lecture will be presented several experiments performed on main photosynthetic antenna complex LHCII (Light Harvesting Complex of Photosystem II) studied in a single molecule regime. The different organization forms of LHCII (monomeric and trimeric) will be discussed in context of light stress conditions and adaptive regulatory mechanisms that operate at the molecular level of this pigment-protein complexes. Applying different excitations it has been noticed, that the photo-protecting mechanism is driven by blue light and operates in the trimeric but not in the monomeric complex of LHCII. The data regarding the intensity and lifetime changes will be presented based on confocal time-resolved microscopy system.

L14.3

Do native and mutated forms of huntingtin interact with human VDAC isoforms?

Andonis Karachitos[1], Daria Grobys[1], Olgierd Stobienia[1], Krzysztof Sobczak[2], Vito De Pinto[3], Hanna Kmita[1]

[1]Laboratory of Bioenergetics, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poznań, Poland; [2]Laboratory of Bioenergetics, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poznań, Poland; [3]Department of Biological, Geological and Environmental Sciences, Section of Molecular Biology, University of Catania, Italy; National Institute for Biomembranes and Biosystems, Section of Catania, Catania, Italy
e-mail: Hanna Kmita <kmita@amu.edu.pl>

Huntington's disease (HD) is an autosomal-dominant neurodegenerative disorder, characterized by a selective loss of neurons mainly from the striatum and deep layers of the cerebral cortex. Clinical symptoms of HD include progressive chorea, rigidity, weight loss, dementia, seizures and psychiatric disturbances such as depression, withdrawal and irritability. Thus, HD gradually robs affected individuals of memory, cognitive skills and normal movements. HD is caused by the mutation of the gene encoding the protein huntingtin (Htt) that results in an increase of above 35 glutamines in the N terminus of Htt (mHtt). Current treatments for HD relieve merely the symptoms but are not able to restore neuronal function nor to stop the insidious loss of neurons. Thus, a new therapeutic approach involving new potential targets and to start before the symptomatic stage could contribute to HD treatment to be more specific and effective.

It is becoming increasingly apparent that mHtt can impair mitochondrial function directly by affecting mitochondrial bioenergetics and dynamics. Simultaneously, it is also evident that the affected phenomena can be modulated by VDAC, directly or by its interaction with the involved proteins. VDAC (voltage-dependent anion-selective channel) is located in the mitochondrial outer membrane and its basic function consists in a transport of metabolites. However, it has been also proved that VDAC is crucial for a range of cellular processes including ATP rationing, Ca^{2+} homeostasis, intracellular redox state regulation, communication between mitochondria and nucleus and apoptosis execution. Thus, the channel is regarded as a dynamic regulator, or even governor, of mitochondrial functions and consequently, of a cell life or death. In mitochondria of different organisms VDAC may be present as isoforms encoded by separated genes, displaying different channel-forming activities and probably playing different roles. *For example,* in human mitochondria, as in the case of other vertebrates, three isoforms of VDAC (hVDAC1-hVDAC3) have been identified. They are expressed in different tissues and organs at different levels.

In our studies, we focused on the interaction of hVDAC1-hVDAC3 with Htt and mHtt. Therefore, we examined the effect of Htt and mHtt on reconstituted hVDAC1-hVDAC3. We also checked the effect of Htt and mHtt expression on hVDAC1-hVDAC3 channel properties using the well known PC12 model of HD. The obtained results indicate that Htt and mHtt directly and differently modulate hVDAC1-hVDAC3. This in turn could be important for development of new therapeutic strategies concerning HD.

Acknowledgements

The studies were supported by the grant: NCN 2011/01/B/NZ3/00359.

Oral presentations

O14.1

Incretin mimetic exendin-4 influence the bioenergetics of human adipocytes

Joanna Góralska[1], Agnieszka Śliwa[1], Monika Chojnacka[1], Anna Polus[1], Anna Gruca[1], Urszula Raźny[1], Małgorzata Kieć-Klimczak[2], Beata Kieć-Wilk[3], Aldona Dembińska-Kieć[1]

[1]Jagiellonian University Medical College, Department of Clinical Biochemistry, Kraków, Poland; [2]Jagiellonian University Medical College, Department of Endocrinology, Kraków, Poland; [3]Jagiellonian University Medical College, Department of Metabolic Diseases, Kraków, Poland
e-mail: Joanna Maria Góralska <j.goralska@uj.edu.pl>

Activation of the NAD+-dependent deacetylases (sirtuins) by small molecules (i.e. polyphenolic compounds), calorie restriction or exercise promotes mitochondrial biogenesis and activities. The switch from energy storage toward increasing energy expenditure in adipocytes is promising method of combating obesity, especially in the context of an impaired ability of adipocytes of obese subjects to aerobic metabolism. The pleiotropic effects of incretins and its mimetics has been partially revealed, but its influence on adipocyte metabolism is not yet completely discovered. The study was aimed to find mechanisms of the pleiotropic influence of GLP-1R agonist, exendin-4, on human adipocytes, with particular emphasis on mitochondria-related effects.

Human cell line CHUB-S7 were differentiated (20 days) to mature adipocytes and than exposed to GLP-1R agonist, exendin-4 (100 nM) or for GLP-1R antagonist, exendin-9 (100 nM) for 24h. Exendin-4 was found to uncouple mitochondrial electron transport from ATP by permeabilizing the inner mitochondrial membrane, slightly decreasing mitochondrial membrane potential analyzed by JC-1 staining and flow cytometry. Basal respiration rate revealed by high resolution respirometry (Oxygraph o-2k, *Oroboros*) in Exendin-4 treated adipocytes was higher than in non treated cells. Moreover, the relative contribution of uncoupled respiration was also significantly higher comparing to control cells. ATP level, determined by luminescent assay, remained unchanged after cells exposure to GLP-1R analog. Intracellular production of Nampt, as well as secretion of visfatin to the culture medium (measured with ELISA method) were higher in exendin-4 treated human adipocytes. The initial analysis of genes expression (Real time PCR) pointed to sirtuins pathway involved in the mitochondrial effects observed. The results were confirmed to be dependent on interaction with GLP-1 receptor, as the presence of exendin-9 abolished the effects of exendin-4.

In conclusion, the stimulatory effects of exendin-4 on mitochondrial bioenergetics, enhancing respiratory capacity and an uncoupled respiration, in mature CHUB-S7 adipocytes was observed. Increasing energy expenditure under the exendin-4 in CHUB-S7 cells suggests a mechanism which may be partially responsible for body weight reduction observed in clinical trials in patients using incretin mimetic drugs in the treatment of type 2 diabetes.

Acknowledgements

Supported by: UJCM grant K/ZDS/004496 and NCN grant no. K/PBN/000001.

O14.2

The influence of STAT proteins on *Dictyostelium discoideum* mitochondrial function

Anna Kicinska, Wieslawa Jarmuszkiewicz

Department of Bioenergetics, Adam Mickiewicz University, Poznań, Poland
e-mail: Anna Kicińska <anias@amu.edu.pl>

STAT (signal transducers and activators of transcription proteins) are one of important mediators of phosphotyrosine-regulated signaling. In multicellular organisms, these proteins are components of signal transduction pathways regulating cellular differentiation, proliferation, immune response, cell fate, cell migration, and programmed cell death. Additionally, members of STAT protein family have been found to be targeted to mitochondria. It has been shown that, in addition to the nuclear transcriptional role, they directly regulate cellular metabolism by influencing mitochondrial function. STAT1 regulates mitophagy, while STAT3 is important for activity of complexes I and II of the mitochondrial electron transport chain. In mitochondria, STAT 5 interacts with E2 component of mitochondrial pyruvate dehydrogenase complex and binds D-loop of mitochondrial DNA. So far, the dual function of STAT (in nuclei and mitochondria) has been found only in mammalian cells. The present work describes a role of STAT proteins in mitochondrial bioenergetics regulation of *Dictyostelium discoideum*. *D. discoideum* is a ameboid protozoon widely used as a model organism in studies of cytokinesis, cell motility, phagocytosis, chemotaxis, signal transduction, and cell differentiation during development. Most of their life *D. discoideum* amoebae undergo a vegetative life cycle as separate, independent cells but, when challenged by starvation, the cells interact to form multicellular structures. *D. discoideum* is one of the simplest organisms that uses STAT-mediated phosphotyrosine-regulated signaling. STAT proteins are important during *D. discoideum* development and hyperosmotic and oxidative stress response. In this study, we show that mitochondria isolated from *D. discoideum* STATC knockout cells differ significantly in their bioenergetics (the activity of electron transport chain complexes) from mitochondria isolated from wild type cells. Knockout cells show 30% lower external NADH dehydrogenase activity, 25% lower succinate dehydrogenase activity, and almost 300% higher complex I activity. Moreover, the activity of NADPH dehydrogenase is significantly higher. The activity of the GMP-stimulated cyanide-resistant alternative oxidase, rotenone-insensitive internal NADH dehydrogenase, and Complex IV remain unchanged compared to wild type mitochondria.

Acknowledgements

This work is founded by Foundation for Polish Science, Pomost/2010-1/3 grant.

O14.3

Live or let die — autophagy analysis in Leber's hereditary optic neuropathy

Agata Kodroń[1], Agata Kulicka[1], Klaudia Karwowska[1], Diana Senator[1], Maciej Krawczyński[3], Magdalena Korwin[4], Biruta Kierdaszuk[5], Anna Kamińska[5], Katarzyna Tońska[1], Ewa Bartnik[1,2]

[1]Institute of Genetics and Biotechnology, University of Warsaw, Warsaw, Poland; [2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; [3]Department of Medical Genetics, K. Marcinkowski University of Medical Sciences, Poznań, Poland; [4]Independent Public Clinical Hospital of Ophthalmology - Department of Ophthalmology, II Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland; [5]Department of Neurology, I Faculty of Medicine, Medical University of Warsaw, Warsaw, Poland
e-mail: Agata Kodron <agatakodron@wp.pl>

In 1988, Leber's hereditary optic neuropathy (LHON) was the first human pathology to be associated with a mitochondrial DNA point mutation. LHON has a markedly reduced penetrance with a clear gender bias. Approximately 50% of men and approximately 10% of women harboring one of the three primary pathogenic mutations develop visual failure. The clinical phenotype of LHON is the degeneration of retinal ganglion cells (RGCs) and a progressive degeneration of the optic nerve. 95% patients with LHON carry one of three mutations in mtDNA – 11778G>A, 3460G>A or 14484T>C. The co-occurrence of two pathogenic mutations responsible for LHON is extremely rare. The pathogenic processes leading to optic nerve atrophy are largely unknown. One of the most common hypotheses explaining the retinal ganglion cell death is that mtDNA mutations increase the level of apoptosis in this tissue. Moreover, other types of cell death could be also involved in retinal ganglion cell degeneration in LHON, for example autophagy.

Autophagy is an evolutionarily conserved homeostatic process for the turnover of cellular contents, organelles and misfolded proteins through the lysosomal machinery. Some scientists believe that autophagy can play a pivotal role in the mechanisms of neurodegenerative disease pathophysiology, therefore in recent years there is more and more research aimed at determining the mechanisms by which autophagy is altered in these disorders.

In 2008 in the Institute of Genetics and Biotechnology a combination of mutation 11778G>A and 3460G>A was found, in a woman and her six children. Both mother and daughter despite a high level of the 11778G>A mutation in the blood had no symptoms characteristic for LHON, but all of the woman's sons were diagnosed with optic nerve atrophy.

Here we present the results of autophagy analysis in different cell lines (fibroblasts, lymphocytes immortalized by EBV virus and transmitochondrial hybrids) derived from 6 patients from a family with a unique combination of two LHON mutations and 6 healthy controls of both sexes. Analysis of autophagy was performed in standard medium and under stress conditions, in medium supplemented with galactose and with rotenone. Differences in autophagy between genders were also investigated to address the question of higher susceptibility to optic nerve degeneration of male carriers of LHON mutations.

O14.4

Functional characteristics of mitochondrial uncoupling protein of human endothelial EA.hy926 cells

Agnieszka Koziel, Wiesława Jarmuszkiewicz

Department of Bioenergetics, Adam Mickiewicz University, Poznań, Poland
e-mail: Agnieszka Koziel <koziel@amu.edu.pl,> Wiesława Jarmuszkiewicz <wiesiaj@amu.edu.pl>

Uncoupling proteins (UCPs) are members of the mitochondrial carrier protein family that are present in the inner mitochondrial membrane and mediate free fatty acid-activated, purine nucleotide-inhibited proton conductance. Functional characteristics of mitochondrial UCP2 in endothelial EA.hy926 cells and isolated mitochondria was studied. We described the UCP2 function in endothelial cells cultured in medium with either a high (25 mM) or normal (5.5 mM) glucose concentration. We studied concentration-dependent effects of free fatty acid, linoleic acid (LA) and purine nucleotide, GTP on endothelial UCP2 activity (i.e., stimulation of mitochondrial respiration and decrease in mitochondrial membrane potential). The UCP2 activity was significantly higher in mitochondria isolated from high glucose-treated cells. An increased expression level of UCP2 in response to high glucose was observed. Reactive oxygen species (ROS) production was measured in endothelial cells and isolated mitochondria. In isolated EA.hy926 mitochondria, we evaluated ROS production when UCP2 was activated by LA and inhibited by GTP. In mitochondria from high glucose-treated cells, a greater LA-induced GTP-inhibited decrease in ROS generation was observed, indicating antioxidative function of UCP2 that was elevated under stress high glucose-induced conditions. Moreover, to examine the role of UCP2 in high glucose-induced oxidative stress of endothelial cells, we silenced UCP2 gene expression using UCP2 siRNA(h) in both types of cells. UCP2 gene silencing led to an elevated rate of mitochondrial ROS formation, especially in high glucose-cultured endothelial cells. Mitochondrial and non-mitochondrial ROS generation was significantly higher in high-glucose cells than in normal-glucose cells independent of UCP2 gene silencing. Moreover, an immunological analysis with antibodies raised against intracellular adhesion molecule-1 (ICAM-1) identified increased inflammation in endothelial cells growth in high glucose concentration, especially in those with UCP2 silenced. In the present study, we described a functional characteristic and antioxidative role of UCP2 in human endothelial cells. Our results indicate that UCP2 may functions as a sensor and negative regulator of mitochondrial ROS production in endothelial cells in response to high glucose.

Acknowledgements

This work is supported by the European Union from the resources of the European Regional Developmental Fund under the Innovative Economy Programme (POIG.01.01.02-00-069/09) and partially the grant from the Polish Ministry of Education and Science (2012/07/N/NZ3/00495).

O14.5

Metabolic violations at alcohol intoxication

Sergei Lelevich[1], Evgeny V. Barkovsky[2]

[1]Grodno State Medical University, Grodno, Belarus; [2]Belarusian State Medical University, Belarus
e-mail: Sergei Lelevich <slelevich@yandex.ru>

In experiment influence of alcohol intoxication of various duration on a condition of a carbohydrate exchange in a liver and skeletal muscles, and also the maintenance of the main neuromediators and neuromediator amino acids in various departments of a brain of rats is studied. Violations of metabolic processes in the studied bodies and the fabrics which expressiveness is defined by doses of entered ethanol and alcoholization duration are revealed. Sharp and chronic alcoholi intoxication leads to violation of activity of key enzymes glicolysis and a pentozofosfate way, and also substrats of a carbohydrate exchange in a liver and skeletal muscles. Violations of a functional condition of dopamine, serotonine and GABA neuromediator systems in a brain are also revealed at alcohol intoxication of various duration.

O14.6

Mitochondrial effect of NS11021 and NS13558 on endothelial cells EA.hy926

Agata Skup[1], Agnieszka Łukasiak[2], Adam Szewczyk[1], Antoni Wrzosek[1]

[1]Laboratory of Intracellular Ion Channels, Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland; [2]Department of Biophysics, Warsaw University of Life Science SGGW, Warsaw, Poland
e-mail: Agata Skup <a.zgrzywa@nencki.gov.pl>

It is well documented that mitochondria play regulatory role in such processes as: apoptosis, reactive oxygen species synthesis and signaling. Recent studies have emphasized the importance of mitochondrial potassium channels in regulation those processes.
It was previously described that activation of large conductance potassium channel (BK_{Ca}) by channel activator, such as NS1619, could protect against ischemia-reperfusion injuries. Although the precise mechanism remains unclear, the experimental evidence suggest that openers of BK_{Ca} have important role in cardioprotection.
In recent years the majority of studies utilized channel activator NS1619. Due to its nonspecific effect on other ion channels, more specific activators of mitochondrial ion channels have been developed. In this study, we investigated the role of BK_{Ca} channel opener NS11021, and its biologically inactive analogue NS13558, in regulation of endothelial function.
As a model of our study we used endothelial immortalized cell line EA.hy926. NS11021, and its analogue NS13558, caused dose-dependent increase oxygen consumption rate in EA.hy926 intact cells and cells permeabilized with digitonin. Along with respiration, mitochondrial potential was measured using fluorescent probe JC-1. NS11021 and NS13558 decreased mitochondrial membrane potential in EA.hy926 mitochondria and this result was similar to CCCP action. We also examined the role of NS11021 and NS13558 in reactive oxygen species (ROS) synthesis using fluorescent probe DCF-DA. We observed that NS11021 decrease ROS level, while NS13558 was ineffective in changing the ROS synthesis level.
These results indicate that in endothelial cells NS11021 and NS13558, besides of BK_{Ca} channel activation, also caused an uncoupling of the proton gradient (similar to CCCP) that is established during the normal activity of electron carriers in the electron transport chain. However, their mechanism of action is still unknown.

Acknowledgements

This work was supported by European Union from the resources of the European Regional Development Fund under the Innovative Economy Programme (POIG.01.01.02-00-069/09).

O14.7

Investigation of mitochondrial dysfunctions in primary fibroblasts derived from ALS patients

Jarosław Walczak[1], Stefan Vielhaber[2], Grażyna Dębska-Vielhaber[2], Jerzy Duszyński[1], Joanna Szczepanowska[1]

[1]Nencki Institute of Experimental Biology, Department of Biochemistry, Warsaw, Poland; [2]University of Magdeburg, Department of Neurology, Germany
e-mail: Jarosław Walczak <j.walczak@nencki.gov.pl>

Impaired mitochondrial functions are involved in the pathophysiology of many neurodegenerative diseases. Amyotrophic Lateral Sclerosis (ALS) is a fatal disorder characterized by progressive and selective degradation of motor neurons responsible for controlling voluntary muscles. Only 10 % of all cases of this disease are recognized as inherited (familial) forms while the rest are sporadic. Defects in mitochondrial energy production and mitochondrial dynamics are early cellular symptoms in ALS development. Understanding the relationship between mitochondrial malfunctions and progress of ALS may provide a useful tool for early diagnosis and potential pharmacological targets for treatment of the disease.
To identify possible mitochondrial stress in fibroblasts derived from patients diagnosed with ALS (familial and sporadic forms) and age matched control subjects, we investigated numerous parameters of mitochondrial physiology. Using living cells we checked mitochondrial membrane potential, ROS and cytosolic calcium levels as well as respiration rates and activity of complex I and IV of electron transport chain. We evaluated level of proteins involved in dynamics of mitochondria (Opa1, Mfn1, Drp1, Fis1), oxidative phosphorylation (ETC complexes), antioxidant protection (SOD1, SOD2) and biogenesis (TFAM).
Control flux coefficient calculated on the basis of titration with specific respiratory inhibitor (sodium amytal) was higher in fibroblasts with sporadic form of ALS while maximal respiration rate on complex I substrates was slightly lowered which may suggest abnormalities in functioning of the complex. The profile of proteins responsible for the dynamics of mitochondria were different in investigated cell lines in comparison to controls. The mitochondrial membrane potential was slightly decreased and cytosolic calcium level was raised in fibroblasts derived from patients with ALS in comparison to controls. We did not notice significant changes in intracellular ROS level.

O14.8

Epigenetics of induced pluripotency: TRIM28, the new kid on the block

Joanna Wróblewska [1,2], Katarzyna Kulcenty [1,2], Urszula Oleksiewicz[1,2], Marta Gładych[1,2], Katarzyna Tomczak[1,2], Sylwia Mazurek[1,2], Parantu Shah[3], Maciej Wiznerowicz[1,2]

[1]Poznan University of Medical Sciences, Chair of Biotechnology, Department of Cancer Immunology, Gene Therapy Laboratory, Poznań, Poland; [2]Greater Poland Cancer Centre, Department of Cancer Diagnostics and Immunology, Gene Therapy Laboratory, Poznań, Poland; [3]Institute for Applied Cancer Science, University of Texas MD Anderson Cancer Center, Houston, TX, USA
e-mail: Joanna Wroblewska <joanna8619@gmail.com>

Human induced pluripotent stem cells (iPS) are generated by reprogramming of somatic cells through enforced expression of embryonic transcription factors. During this process the genome undergoes major chromatin remodeling, acquiring euchromatic state, characteristic for pluripotent cells. TRIM28 is one of epigenetic regulators involved in changes of chromatin structure.
To fully understand the role of TRIM28 in reprogramming and self-renewal process, we first developed protocol to generate iPS cells from human fibroblasts. Transduction of target cells with lentiviral vector pSTEMCCA-tetO allowed for ectopic expression of reprogramming factors OCT4, SOX2, KLF4 and cMYC and thus efficient generation of human iPS clones. Obtained clones were picked and further cultured until establishing stable iPS cell lines. Then cells were transduced with another vector pLV-HK carrying tTRKRAB, which binds to tetO element, in order to switch off reprogramming transgene expression. Tight repression of introduced transgenes in all human IPS clones was analyzed by RT-PCR and confirmed full functionality of our system. Obtained IPS cell lines showed no abnormalities in karyotypes. Pluripotent phenotype of iPS cells was confirmed by several analyzes including embryonic gene expression, formation of embryonic bodies *in vitro* and teratomas *in vivo*. Genomic integrity and homogeneity of generated iPS cell lines was confirmed by RNA-seq. tTRKRAB-mediated epigenetic repression persisted through prolonged culture of obtained iPS cell lines.
To probe the role of TRIM28 in reprogramming process, we generated TRIM28-knockdown fibroblasts, and then submitted them to reprogramming with described protocol. Our results showed, that TRIM28 knockdown accelerates the emergence of iPS colonies at early stages of reprogramming process. TRIM28 knockout cells showed higher expression of markers characteristic for pluripotent state. To confirm these observations, RNA-seq analysis were performed. Our results implicate, that epigenetic mechanisms controlled by TRIM28 protein are involved in the reprogramming process. The second aspect of analyzing role of TRIM28 in reprogramming was to examine its impact on the global methylation profile. For this purpose, the DNA material was subjected to global methylation analysis using RRBS technique. To probe the role of TRIM28 in self-renewal process, we silenced its expression with RNA interference reagent. Our results showed that after around 14 days, TRIM28-knockdown iPS cells lost morphology typical for pluripotent cells. Along with loss of morphology, we observed decrease in pluripotency markers expression and increased expression of differentiated cells markers.
Our research proved that that epigenetic mechanisms controlled by TRIM28 protein works as an barrier in reprogramming process, but is necessary in iPS cells to maintain their pluripotent state.

Posters

P14.1

The TOB/SAM complex of the slime mold *Dictyostelium discoideum* at the vegetative stage of its life cycle

Monika Antoniewicz[1], Olgierd Stobienia[1], Małgorzata Słocińska[2], Dorota Raczyńska[3], Hanna Kmita[1], Małgorzata Wojtkowska[1]

[1]Institute of Molecular Biology and Biotechnology, Department of Bioenergetics, Adam Mickiewicz University, Poznań, Poland; [2]Institute of Experimental Biology, Department of Animal Physiology and Development, Adam Mickiewicz University, Poznań, Poland; [3]Institute of Molecular Biology and Biotechnology, Department of Gene Expression, Adam Mickiewicz University, Poznań, Poland
e-mail: Monika Antoniewicz <monika.a@amu.edu.pl>

The TOB/SAM complex (translocase of the outer membrane β-barrel proteins/sorting and assembly machinery) is located in the mitochondrial outer membrane and is required for the insertion of β-barrel proteins into the membrane. Mitochondrial β-barrel proteins participate for example in protein import (e.g. Tom40) or metabolite transport (e.g. VDAC). It is also suggested that the TOB/SAM complex provides a flexible platform to integrate the sorting pathways of different precursor proteins and to promote their assembly into the complexes. Therefore the TOB/SAM complex appears to have a fundamental meaning for proper function of mitochondria and consequently for a cell functioning. Interestingly, differences in the TOB/SAM complex subunit organization has been shown for representatives of different phylogenetic lineages. Additionally, within mitochondria of a given organism different forms of the TOB/SAM complex may occur. The slime mold *Dictyostelium discoideum* is known as a valuable research model for developmental biology and medicine because its life cycle offers a big variety of unicellular and multicellular stages as well as multiple cell types to study. Here we report our results concerning the TOB/SAM complex of myxamoebae of *D. discoideum*, i.e. its unicellular form dividing intensively. The molecular weight of the isolated complex range between 250-350 kDa and the complex appears to be present in two forms. After reconstitution in black lipid membranes it displays a channel activity of a characteristic described for the TOB/SAM complexes of model organisms. The complex contains Tob55/Sam50 being a core subunit of the complex and a putative second subunit that reveals sequence similarity to human metaxin. Interestingly, using antibody against Tob55/Sam50 N-terminus we observe two forms of the protein in mitochondria. Accordingly, there are two products of PCR with fluorescent probe labeling that suggests the presence of two splicing forms. Further studies of the TOB/SAM complex subunit organization in cell representing different stages of *D. discoideum* life cycle are important to obtain data concerning the involvement of the complex in processes decisive for a cell functional state.

Acknowledgements

The studies were supported by NCN "PRELUDIUM" UMO-2012/05/N/NZ3/00293.

P14.2

Functional expression of mitochondrial uncoupling protein of *Acanthamoeba castellanii* in yeast *Saccharomyces cerevisiae*

Nina Antos-Krzeminska, Wieslawa Jarmuszkiewicz

Adam Mickiewicz University, Faculty of Biology, Department of Bioenergetics, Poznań, Poland
e-mail: Nina Antos-Krzemińska <nant@wp.pl>

Uncoupling proteins (UCPs) belong to the inner mitochondrial membrane anion transporters. They uncouple the oxidation of fuels via the electron respiratory chain from ATP synthesis by mediating of proton leak into mitochondrial matrix. Theferore, UCPs dissipate energy as heat and affect energy cellular metabolism. A direct consequence of the free fatty acid-stimulated UCP activity is a decrease in the oxidative phosphorylation efficacy. Recently, gene encoding uncoupling protein in free-living protozoan *Acanthamoeba castellanii* (AcUCP) has been identified. The gene was amplified and cloned to yeast expressing pYES2 vector. Yeast *Saccharomyces cerevisiae* do not possess UCP, so they provide an excellent model to confirm functionally AcUCP gene's product presence. We transformed InvSc1 strain of *S. cerevisiae* with pYES2+AcUCP vector and confirmed the proper transformation by selection of mutants in the medium without uracil. The presence of heterologously introduced gene was proved by PCR amplification of AcUCP in yeast. Moreover, we detected AcUCP in transformed yeast cells after D-galactose induction, using antibody raised again human UCP3. In non-phosphorylating AcUCP-transformed yeast mitochondria, functional studies showed a linoleic acid-induced purine nucleotide-inhibited uncoupling, which can be attributed to AcUCP activity.

Acknowledgements

This work is supported by the grant from the Polish Ministry of Education and Science (NN301636440).

P14.3

Identification potassium channels in the skin mitochondria

Piotr Bednarczyk[1,2], Adam Szewczyk[2]

[1]Warsaw University of Life Sciences - SGGW, Department of Biophysics, Warsaw, Poland; [2]Nencki Institute of Experimental Biology, Laboratory of Intracellular Ion Channels, Warsaw, Poland
e-mail: Piotr Bednarczyk <piotr_bednarczyk@sggw.pl>

Potassium channels have been found in the inner mitochondrial membranes of various cells. The activation of these channels is cytoprotective. Hence, the identification of ion channels present in the inner mitochondrial membrane of skin cells (keratinocytes and fibroblasts) is important in distinguishing possible protective mechanisms in these cells. In our study, inner membrane mitochondrial ion channels of the human keratinocyte and fibroblast cells were investigated using a patch-clamp technique. In the inner mitochondrial membrane of fibroblast we detected large-conductance Ca^{2+}-regulated potassium channel (mitoBK$_{Ca}$ channel) and also activity of the ATP-regulated potassium channel (mitoK$_{ATP}$ channel). In keratinocytes mitochondria we observed potassium-selective channel activity with a conductance of 83 pS at positive voltages. The shape of the current curve indicates that the observed channel has rectifying properties. Moreover, the channel activity was inhibited by acidic pH and 1 mM lidocaine. Using reverse transcriptase, we found an mRNA transcript for the tandem pore domain acid-sensitive potassium channel (TASK-3 channel). Additionally, we showed that TASK-3 channel knockdown of keratinocyte cells markedly decreased viability after UVB radiation exposure compared with control cells.

Our findings indicate presence of the potassium channels in the inner mitochondrial membranes of human skin and their possible role in protection.

Acknowledgements

This study was supported by a grant MERIS PBS1/B8/1/2012 from the National Centre of Research and Development.

P14.4

Glycogen metabolism in cells with glycogen branching enzyme deficiency

Małgorzata Bejtka, Jerzy Duszyński, Dominika Malińska

Nencki Institute of Experimental Biology, Department of Biochemistry, Warsaw, Poland
e-mail: Małgorzata Bejtka <mbejtka@nencki.gov.pl>

Glycogen branching enzyme (GBE1) deficiency leads to a genetic disease: glycogen storage disease type IV (GSD IV), affecting mostly the liver, skeletal muscles and the nervous system. It is a very rare, autosomal recessive disorder. Its severity, age of onset and symptoms depend on the specific mutation a patient has.

The mutation-dependent deficiency of GBE1 results in intracellular accumulation of poorly branched glycogen molecules, which have lower solubility and may lead to mechanical cell damage, slower glucose release from such glycogen deposits, and – as a consequence – abnormal intracellular energy metabolism. It is not clear to which extent the cell malfunction is an effect of energy metabolism disruption or of mechanical damage to cells.

In our study we work with primary human skin fibroblasts from patients with classical (childhood) and adult form of the disease. We have compared patient and control cells in terms of GBE1 level, glycogen content, glycogen dynamics and branching. In both GSDIV models we find that the cells have a lower level of GBE1. At the same time the accumulation of glycogen is higher than in controls, even though glycogen synthase level remains unchanged. In stress induced conditions, this accumulation becomes even higher. In culture under starvation, patient cells are able to mobilize glycogen to the same extent as controls.

P14.5

Cell viability and distribution of huntingtin in PC12 model of Huntington disease under conditions affecting cell division, mitochondrial bioenergetics and mitochondrial dynamics

Daria Grobys[1], Andonis Karachitos[1], Tomasz Skrzypczak[2], Hanna Kmita[1]

[1]Institute of Molecular Biology and Biotechnology, Departament of Bioenergetics, Adam Mickiewicz University in Poznan, Poznań, Poland; [2]Institute of Molecular Biology and Biotechnology, Departament of Molecular and Cellular Biology, Adam Mickiewicz University in Poznan, Poznań, Poland
e-mail: Daria Grobys <grobysdaria@gmail.com>

Huntington disease (HD) is an autosomal-dominant neurodegenerative disorder characterized by a selective loss of neurons. HD is caused by a CAG trinucleotide repeat expansion in exon 1 of *IT15* gene encoding huntingtin (Htt). As the trinucleotide codes for glutamine, its repeat number higher than 35 results in an abnormally long polyglutamine tract in Htt N terminus that gives rise to its mutated form (mHtt). It is now obvious that mitochondria play a vital role in HD pathogenesis. Moreover, available data indicate that mitochondrial defects initiate disease onset. At present it is proposed that both impairment of bioenergetic aspects of mitochondria functioning as well as mitochondrial fusion and fission (also termed mitochondrial dynamics) are early and crucial events in HD pathogenesis. However, that cause-effect relationships between mitochondrial dynamics and bioenergetics is still not explain. It is suggested that mitochondrial dynamics can be regulated by the mitochondrial energy status or can control the status. Therefore study of relationships between mitochondrial energy status and dynamics appears to be a logical step in elucidation of mitochondrial dysfunction in HD pathomechanism

We applied PC12 cell lines derived from rat pheochromocytoma, namely PC-12HD-Q23 and PC-12HD-Q74[1]. The cells express *IT 15* exon 1 containing 23 or 74 repeats of glutamine codon that results in expression of Htt and mHtt, respectively. The expression was induced by doxycycline, and monitored due to GFP labeling. PC12 cycling cells were differentiated into post-mitotic neuron-like cells upon treatment with the nerve growth factor (NGF). This enabled studies on cells differing in division activity and level of differentiation. We also used media with different concentration of glucose to affect mitochondrial energy status within intact cells as well as Mdivi-1, an inhibitor of mitochondrial fission, to estimate the impact of mitochondrial fission. The analysis of protein distribution and cell viability under the applied conditions was performed by confocal microscopy. The obtained results indicate that the presence of NGF and Mdivi-1 distinctly influences the levels of Htt and mHtt expression and distribution as well as viability of cells. Therefore they contribute to understanding of mitochondria role in HD etiology.

Reference:

1. Wyttenbach A *et al* (2001) *Human Molecular Genetics* **10**: 1829–1845.

Acknowledgements

The studies were supported by the grant: NCN 2011/01/B/NZ3/00359
The cells were obtained from David Rubinsztein and Andreas Wyttenbach from Cambridge University.

P14.6

Activation of two mitochondrial energy-dissipating systems, uncoupling protein and ATP-regulated potassium channel decreases superoxide anion production in insect mitochondria

Małgorzata Slocinska[1], Grzegorz Rosiński[1], Wieslawa Jarmuszkiewicz[2]

Adam Mickiewicz University, [1]Departament of Animal Physiology and Development; [2]Department of Bioenergetics, Adam Mickiewicz University, Poznań, Poland
e-mail: Wieslawa Jarmuszkiewicz <wiesiaj@amu.edu.pl>

Uncoupling protein (UCP) and mitochondrial ATP-regulated potassium channel (mitoK$_{ATP}$ channel) are proteins located in the inner mitochondrial membrane. Recently, we have showed that these energy-dissipating systems are present in insect tissues such as trophic tissue of fat body and leg muscle. In cockroach *Gromphadorhina coquereliana*, UCP4 as well as mitoK$_{ATP}$ channel decrease superoxide anion production. In the present study we elucidated whether UCP4 and mitoK$_{ATP}$ channel collaborate in modulating of reactive oxygen species production in fat body and muscle of the cockroach. In isolated mitochondria, UCP was activated by palmitic acid and mitoK$_{ATP}$ channel was stimulated by diazoxide or pinacidil and superoxide anion formation was measured by nitroblue tertazolim method. Simultaneous activation of both proteins resulted in a significant decrease in superoxide level that was much stronger when the proteins were activated separately. After addition of the UCP and mitoK$_{ATP}$ channel inhibitors (GTP and/or ATP), the level of superoxide significantly increased, but it was still almost twice lower compared to control conditions (no activators and inhibitors). When both UCP and mitoK$_{ATP}$ channelwere activated in the presence of GTP, the observed increase in superoxide production was higher compared to measurements with ATP. These results suggest a greater amount of UCP protein in insect inner mitochondrial membrane and thus a more significant role of this protein in modulation of mitochondrial reactive oxygen species formation. The cumulative effects of UCP4 and mitoK$_{ATP}$ channel activation on cockroach mitochondrial superoxide formation indicate a physiological role of both proteins in modulating reactive oxygen species formation in insect fat body and muscle. We hypothesize that insect UCP4 and mitoK$_{ATP}$ channel might be implicated in a cellular protection against metabolic stress during energy demand events such as molting or flight.

Acknowledgements

This work is supported by the grant from the Polish Ministry of Education and Science (NN301636440).

P14.7

Measurements of VDAC conductance in the presence of huntingtin proteins

Andonis Karachitos[1], Olgierd Stobienia[1], Krzysztof Sobczak[2], Vito De Pinto[3,4], Hanna Kmita[1]

[1]Laboratory of Bioenergetics, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poznań, Poland; [2]Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poznań, Poland; [3]Department of Biological, Geological and Environmental Sciences, Section of Molecular Biology, University of Catania, Italy; [4]National Institute for Biomembranes and Biosystems, Section of Catania, Catania, Italy
e-mail: Andonis Karachitos <andonis@amu.edu.pl>

Huntington's disease (HD) is an autosomal-dominant neurodegenerative hereditary disorder that gradually robs affected individuals of memory, cognitive skills and normal movements. It is originated by the mutation of the gene encoding the huntingtin-protein (Htt). Htt with an abnormal stretch of above 35 glutamines in the N terminus (mHtt) results in HD. The observed symptoms correlate with the selective loss of neurons within the central nervous system, mainly in the striatum but also in the cerebral cortex.
It is also evident from some of studies that the severity of mitochondrial abnormalities correlates with increasing number of glutamine repeats in mHtt, suggesting mitochondria as a major target of mHtt. The proposed mitochondrial targets of mHtt include processes that are known to be affected by VDAC (voltage-dependent anion selective channel), i.e. the respiratory chain, protein import, calcium balance, oxidative stress, transcription regulation, communication between mitochondria and nucleus and apoptosis. In mitochondria of different organisms VDAC may be present as isoforms encoded by separated genes, displaying different channel-forming activities and probably playing different roles. *S. cerevisiae* mitochondria express two VDAC isoforms (yVDAC1 and yVDAC2), of which only yVDAC1, encoded by *POR1* gene, has been proved to form a channel with properties highly conserved in other species. In human mitochondria, as in the case of other vertebrates, three isoforms of VDAC (HVDAC1-HVDAC3) able to form functional channels have been identified.
Here we report our results concerning the effect of Htt and mHtt on a channel activity of human VDAC isoforms reconstituted in a planar lipid bilayer (BLM system). The obtained results indicate that Htt and mHtt directly and differently affect the channel activity of human VDAC isoforms.

Acknowledgements

The studies were supported by the grant: NCN 2011/01/B/NZ3/00359.

P14.8

Cytoprotective role of TASK-3 in the Mitochondria of Human Keratinocytes

Michal Laskowski[1], Renata Toczylowska-Maminska[2], Anna Olszewska[1], Piotr Bednarczyk[1,2], Krzysztof Skowronek[3], Adam Szewczyk[1]

[1]Nencki Institute of Experimental Biology, Laboratory of Intracellular Ion Channels, Department of Biochemistry, Warsaw, Poland; [2]Warsaw University of Life Science, Department of Biophysics, Poland; [3]International Institute of Molecular and Cell Biology, Laboratory of Bioinformatics and Protein Engineering, Warsaw, Poland
e-mail: Michal Laskowski <m.laskowski@nencki.gov.pl>

The activation of mitochondrial potassium channels induces cytoprotection in various cell types.Inner membrane mitochondrial ion channels of the human keratinocyte HaCaT cell line wereinvestigated using a patch-clamp technique, reverse transcriptase–PCR and immunochemistry toconfirm presence of TASK-3 channel. We showed that TASK-3 knockdown HaCaT cells markedlydecreased viability after UVB radiation exposure compared with control cells.

P14.9

Mitochondria-shaping proteins in cells derived from patients with Alzheimer's and Parkinson's diseases

Małgorzata Partyka[1], Grażyna Dębska-Vielhaber[2], Stefan Vielhaber[2], Jerzy Duszyński[1], Joanna Szczepanowska[1]

[1]Nencki Institute of Experimental Biology, Department of Biochemistry, Warsaw, Poland; [2]Otto-von-Guericke University Magdeburg, Department of Neurology, Magdeburg, Germany
e-mail: Malgorzata Partyka <m.partyka@nencki.gov.pl>

Mitochondrial shape constantly changes in accordance with variations of cellular requirements. Control of mitochondria dynamics is essential for maintaining their proper functioning.
Mitochondrial dynamics is regulated by mitochondrial-shaping proteins. Fission proteins (Drp1, Fis1) are responsible for the transport and segregation of mitochondrial fragments during cell division, as well as for removal of malfunctioning fragments of the network. Mitofusins (Mfn) and Opa1 proteins are responsible for mitochondrial fusion, which is needed to maintain correct metabolic and respiratory activity of mitochondria.
Numerous studies have revealed that disturbances of mitochondrial dynamics are implicated in neurodegenerative disorders. Our purpose is to investigate this process in primary cultures of skin fibroblasts derived from patients suffering from sporadic Alzheimer's and Parkinson's diseases (sAD and sPD).
We studied levels and modifications of proteins responsible for mitochondrial dynamics. Total level of fission protein Drp1 was increased only in sPD patients cells, but the level of Drp1 fission promoting Ser-616 phosphorylated form was increased also in sAD fibroblasts.
The levels of mitochondrial fusion proteins (Opa1, Mfn1, Mfn2) were different in patients than in control cells.
Organization of mitochondria was to certain extent different in patients fibroblasts than in the control cells.
To characterize the mitochondrial metabolism and bioenergetic parameters in sAD and sPD fibroblasts we study parameters of mitochondrial metabolism such as intracellular ROS and calcium levels, mitochondrial membrane potential, mitochondrial mass and level of ATP in the fibroblasts. Mitochondrial membrane potential is decreased in sAD and sPD fibroblasts and mitochondrial mass is lower in sPD patients cells in comparison to control.

P14.10

Research of metabolic pathway of 4-pyridone-3-carboxamide-1β-D-ribonucleoside – studies with participation of Human Embryonic Kidney 293 cell line

Iwona Pelikant-Małecka, Alicja Sielicka, Ewa Kaniewska, Adrianna Radulska, Ryszard T. Smoleński, Ewa M. Słomińska

Medical University of Gdansk, Department of Biochemistry, Gdańsk, Poland
e-mail: Iwona Pelikant-Małecka <iwpe-ma@gumed.edu.pl>

We identified that endogenously produced nucleoside 4-pyridone-3-carboxamide-1β-D-ribonucleoside (4PYR), a compound related to nicotinamide give raise to a family of nucleosides phosphates – 4PYTP, 4PYMP and NAD related derivative 4PYRAD. These derivatives could be formed in all types of cells. Previous studies demonstrated that formation of 4PYMP and 4PYTP was mediated by adenosine kinase activity. Pathway of 4PYRAD formation has not yet been identified. However there is evidence that this process involves 4PYMP as a intermediate product. The aim of this study was to analyze whether adenosine kinase (AK), cytosolic 5'nucleotidase type II (NT5C2) and nicotinamide nucleotide adenylyltransferase type III (NMNAT3) enzymes were involved in metabolic pathway of 4PYR.

Methods: HEK 293T cells in which 4PYR is metabolized to 4PYMP and 4PYRAD (after 48h treatment with 100uM 4PYR) were used in this study. siRNA for AK, NT5C2, NMNAT3 genes and non-targeting negative control siRNA were purchased (Qiagen). For the experiments HEK 293T cells were plated in 24-well plates. Next day medium was removed and cells were washed with Opti-MEM® + GlutaMAX™ (Gibco). After that to each well Opti-MEM® + GlutaMAX™ was added. In this condition cells were transfected according to the manufacturer's instruction using Lipofectamine RNAiMAX reagent (Invitrogen). Cells were transfected with a final concentration of 6 pmol siRNA for each well. After 24 hours medium was removed and fresh full suplemented DMEM medium with addition of 100 μM 4PYR was added. Cells transfected with AK siRNA were supplemented with 100 μM adenine and 2.5 mM ribose to provide alternative substrates to maintain adenine nucleotide pool. After 48 hour of incubation, cells and incubation medium were separated and cellular 4PYMP and 4PYRAD were measured by HPLC. Protein precipitates were dissolved and analyzed with Bradford method.

Results: Transient silencing of AK resulted in reduction of 4PYMP and 4PYRAD concentration to less than 25% of control value with negative control siRNA. Silencing of NT5C2 resulted in four fold increases of PYMP compared to control while 4PYRAD level was not different. After transient silencing of NMNAT3 4PYRAD concentration decreased to half of control level while 4PYMP concentration remained the same.

Conclusion: Our results suggests that adenosine kinase, cytosolic 5'nucleotidase II and nicotinamide nucleotide adenylyltransferase III are involved in 4PYR metabolism. However other forms of cytosolic 5'nucleotidase and nicotinamide nucleotide adenylyltransferase should be considered as a potential enzymes involved in 4PYR metabolism

Acknowledgments

This study was supported by European Union from the resources of the European Regional Development Fund under the Innovative Economy Programme (grant coordinated by JCET-UJ, No POIG.01.01.02-00-069/09) and National Science Centre of Poland (2011/01/B/NZ1/01629).

P14.11

Drp1 protein mobility in the living cell and its interactions with mitochondria: quantitative analysis

Bernadeta Pyclik, Paulina Patalas, Jerzy Duszyński, Jędrzej Szymański

Nencki Institute of Experimental Biology PAS, Warsaw, Poland
e-mail: Bernadeta Pyclik <b.pyclik@nencki.gov.pl>

Living cell consists of a huge number of biomolecules that function in a very dynamic and complex environment. Proteins in the cell are in continuous movement and undergo various interactions. These different kinds of protein activities are usually studied *in vitro*. But complex environment in the living cell is different from buffer solutions conditions. This is the gap which we want to fill by doing quantitative analysis of Drp1 protein movement and interactions in the living cell.

Drp1 protein is involved in the mitochondrial fission. Upon fission signals in the cell, Drp1 monomers form oligomeric ring-like structure which wraps around mitochondria and then constricts thanks to the energy gained from GTP hydrolysis. This constriction results in mitochondrial fragmentation. Precise state of Drp1 protein population in the cytosol is not yet known. So far we do not exactly know if this protein stays in cytoplasm in dimeric, tetrameric or any other state.

Method, we use to quantify Drp1 movement, extent of aggregation and interactions in the living cell, is Fluorescence Correlation Spectroscopy. This technique allows to measure the diffusion coefficient of the GFP-tagged Drp1 moving through defined confocal volume. Since diffusion coefficient is inversely proportional to the object size, the estimate of Drp1 oligomeric state in the cytosol can be obtained from such measurement. Obtained results will help to assess Drp1 fraction in the cytosol and at the mitochondria. We will also be able to answer the question if Drp1 form oligomers in the cytosol or oligomerizes only at the mitochondrial surface.

Acknowledgements

Research grant from the Polish National Science Centre (funds granted based on the decision number DEC-2013/08/W/NZ1/00687).

P14.12

Mitochondria-related effects induced by free fatty acids in pancreatic beta cells and human preadipocytes

Agnieszka Śliwa[1], Joanna Góralska[1], Beata Kieć-Wilk[2], Anna Polus[1], Anna Gruca[1], Aldona Dembińska-Kieć[1]

[1]Jagiellonian University Medical College, Department of Clinical Biochemistry, Kraków, Poland; [2]Jagiellonian University Medical College, Department of Metabolic Diseases, Kraków, Poland
e-mail: Agnieszka Śliwa <a.sliwa@uj.edu.pl>

Western diet is characterized by high content of fats and carbohydrates and a limited intake of plant products. Nutrient overload (increased free fatty acids and glucose intake) leads to metabolic disorders such as obesity, insulin resistance and type 2 diabetes. FFAs are more than substrates for oxidative metabolism playing a significant role in cell signaling, they influence – cell membrane fluidity, gene expression, ion homeostasis, etc. Excess of fatty acids accompanied by triglyceride accumulation in multiple cells including adipocytes, hepatocytes, skeletal muscle, pancreatic beta cells and fibroblast results in chronic cellular injury, low grade inflammation and dysfunction. FFAs increase mitochondrial generation of ROS by depolarization of the mitochondrial inner membrane due to the uncoupling effect.

The aim of the study was to investigate the effects of selected free fatty acids on mitochondrial function in murine pancreatic beta cells and human preadipocytes.

Methods: As an experimental model mouse beta cells (BTC6) line and immortalized human preadipocyte cell line (Chub-S7) was selected. The cells were incubated for 24h with different concentration (30–600 uM) of FFAs (PA, AA, EPA). Chub-S7 cells were incubated with lower concentration of FFAs than BTC-6, because of the higher susceptibility to apoptosis of these cells. Mitochondrial function was monitored by measurements of the mitochondrial oxygen consumption (high-resolution respirometry, OROBOROS Oxygraph-2k), ATP content (luminescence method, Parkin Elmer), mitochondrial membrane potential (JC-1 dye, flow cytometry BD), activity of the caspase-9 (colorimetric method, R&D Systems) and ROS generation (fluorescence method, DCFH-DA).

Results: In preadipocytes: PA impaired mitochondrial function that was manifested by decreased intracellular ATP content and increased ROS generation. AA acid increased ROS generation and caspase-9 activity related with the mitochondrial apoptosis pathway. EPA decreased mitochondrial respiration and ATP content. In BTC6 line AA slightly increased mitochondrial respiration as well as EPA increased the amount of early apoptotic cells. There was also tendency to increased caspase-9 activity after incubation with FFAs.

In conclusion, free fatty acids differentially affect mitochondrial function of selected adipose and pancreatic cell lines, but activate proapoptotic pathways.

Acknowledgements

Supported by K/PBN/000001, K/ZDS/004496, K/ZDS/002286, K/ZDS/002333 grants.

P14.13

The influence of palmitic acid on the aerobic metabolism of human endothelial cells

Izabela Sobieraj, Wieslawa Jarmuszkiewicz

Department of Bioenergetics, Adam Mickiewicz University, Poznań, Poland
e-mail: Izabela Sobieraj <izabela.sobieraj@amu.edu.pl>

Endothelium is considered to be slightly dependent on the mitochondrial energy supply. An excess of free fatty acids in plasma can contribute to the endothelial dysfunction inducing oxidative stress, apoptosis and inflammatory response.The aim of the study was to examine mitochondrial respiratory functions in endothelial cells and to assess the influence of the free fatty acid exposure on their viability and function.

Human umbilical vain endothelial cell line (EA.hy926) was cultured in a medium containing different concentrations (100–400 μM) of palmitic acid. The cell response was observed after a short (12 h, 24 h and 48 h) or a chronic (6 days) exposure to palmitic acid. To find an optimal concentration of the applied free fatty acid, which causes an inflammatory effect, a level of protein expression of intercellular adhesion molecule 1 (ICAM1) was determined. In general, the longer time the endothelial cells were treated with a high concentration of palmitic acid, the higher expression level of ICAM 1 was, indicating elevated inflammatory response. The oxygen consumption rate (OCR) was measured for endothelial cells using the Clark-type electrode. We estimated the maximal OCR that the cells can sustain, ATP-linked OCR and non-ATP-linked OCR components of the basal respiratory rate in untreated and palmitic acid-treated cells. The respiratory response to elevated palmitic acid was observed in cells grown in 100 or 150 μM palmitic acid for at least 6 days or longer. To examine how endothelial cells grown under control and high palmitic acid conditions respond to simply change in respiratory substrates, mitochondrial respiratory function was measured with the glycolytic (glucose, pyruvate), lipid (palmitic acid) and amino acid (glutamine) fuels. Protein expression of markers of different catabolism pathways was studied. Moreover, high palmitic acid conditions produced increased mitochondrial and non-mitochondrial ROS formation, leading to decreased cell viability.

Acknowledgements

This work is supported by the European Union from the resources of the European Regional Developmental Fund under the Innovative Economy Programme (POIG.01.01.02-00-069/09).

P14.14

Morphine and alcohol withdrawal cause adaptive changes in GABA catabolism in rat brain structures

Hanna Vinitskaya, Vladimir Lelevich

Department of Biochemistry[1], Central Research Laboratory[2], Grodno State Medical University, Grodno, Belarus
e-mail: Hanna Vinitskaya <vinhanna3310@gmail.com>

Protracted consumption of narcotic drugs evokes significant metabolic changes in a body, resulting in the development of physical dependence. Evidence from recent studies increasingly points to the brain GABAergic system as being of fundamental importance in mediation of alcohol and opiate withdrawal signs, either at a receptor level or due to its metabolic role in the brain. The gamma-aminobutyrate (GABA) shunt, an alternative route for the conversion of alpha-ketoglutarate to succinate, involves the glutamate decarboxylase, the GABA transaminase (GABA-T) and the succinate semialdehyde dehydrogenase (SSA-DH). Several lines of research suggest a close relation between minor GABA-shunt and the brain carbohydrate and energy metabolisms, and GABA-shunt was hypothesized to be an additional deliverer of Krebs' cycle substrates and a corrector of the disturbances caused by some toxic substances (alcohol, opioids, pentachloronaphtalenes, etc).
The aim of the study was to compare the effects of alcohol and morphine withdrawal (AW and MW) on the activities of GABA-T and SSA-DH and the TCA cycle enzymes (succinate dehydrogenase, NAD^+-dependent isocitrate dehydrogenase (NAD^+-IDH)) in the brain regions of rats (cortex, brain stem).
In aw model rats the 5 days' chronic alcohol intoxication and cessation caused in cortex the reliable increase in the GABA-T activity 3 hours after AW. The NAD^+-IDH activity was higher after 1, 3 and 7 days of AW showing the higher rate of Krebs' cycle after alcohol cessation, whereas the GABA-T activity decreased in the same groups.
Cessation of morphine after preliminary protracted drug exposure induced changes in the indices tested, which were highly dependent upon the term of MW and varied in the brain regions tested. MW led to the increased GABA-T activity in cortex and brain stem, indicating higher GABA turnover. The NAD^+-IDH activity has higher in cortex and decreased in brain stem in all terms of MW.
The results obtained support the notion that dependence upon narcotic drugs might develop via similar neurochemical mechanisms. It was proposed that the changes observed may occur due to non-specific adaptation of neurons to the excessive drug intake and further withdrawal. The elucidation of the mechanisms that underlie adaptations to alcohol and morphine intoxication are leading to a better understanding of the implication of the brain GABA system and new targets for therapies to support recovery from drug withdrawal and addiction.

P14.15

A case report of the amoeba *Acantahmoeba castellanii* and the slime mold *Dictyostelium discoideum* TOM complex

Małgorzata Wojtkowska[1],Olgierd Stobienia[1], Monika Antoniewicz[1], Dorota Buczek[1,2], Aleksandra Gryciuk[1], Wojciech Makałowski[2], Hanna Kmita[1]

[1]University of Adam Mickiewicz, Faculty of Biology, Institute of Molecular Biology and Biotechnology, Poznań, Poland; [2]University of Münster, Institute of Bioinformatics, Münster, Germany
e-mail: Małgorzata Wojtkowska <woytek@amu.edu.pl>

The import efficiency of the most preproteins into mitochondria depends on the wide variety of proteins forming complexes localized in both mitochondrial membranes. One of these complexes is a preprotein translocase known as the TOM complex (translocase of the outer membrane) and regarded as the main gate into mitochondria for imported proteins. Namely, the complex is responsible for decoding of targeting signals, translocation of imported proteins across or into the outer membrane, and their subsequent sorting. Thus, the TOM complex appears to be fundamental for mitochondrial functioning. It has been proposed that protein import complexes, including the TOM complexes, are of modular construction. It means that each complex contains core module formed by proteins common to all eukaryotes and additional modules that have been added over time and regarded as common only to a particular eukaryotic lineage. Available data indicate that indeed mitochondria of representative organisms from across the major phylogenetic lineages of eukaryotes differ in subunit organization of the TOM complex. As forthe representative ofAmoebozoa the subunit organization of the TOM complex is still discussed we decided to estimate the organization for the amoeba *Acanthanoeba castellanii* and the slime mold *Dictyostelium discoideum*, representatives of Amoebozoa two major subclades, namely Lobosa and Conosa, respectively. The obtained results indicate that both complexes contain Tom40 and Tom7 while other subunits are different. The *A. castellanii* TOM complex contain also homologues of Tom20, Tom22 and Tom70 of fungi and higher eukaryotes. Unexpectedly, for the *D. discoideum* TOM complex we were able to find only not known but undoubtedly associated with the purified complex proteins of a molecular weight of approximately 9, 30 and 60kD. Taken together representatives of two major subclades of Amoebozoa differ significantly in organization of the TOM complex that probably reflect the differences in their life cycle and environment requirements.

P14.16

Revision of GDP inhibitory effect on fatty acid-induced UCP-mediated proton leak in isolated mammalian mitochondria

Andrzej M. Woyda-Ploszczyca, Wieslawa Jarmuszkiewicz

Adam Mickiewicz University, Faculty of Biology, Department of Bioenergetics, Poznań, Poland
e-mail: Andrzej Marek Woyda-Ploszczyca <awoy@amu.edu.pl>

We postulate that GDP could not function as a potent inhibitor of mitochondrial uncoupling protein (UCP) based on observation that a high concentration of GDP (1 mM) stimulates respiratory rate and decreases membrane potential of mammalian (rat kidney and human endothelial cells) isolated mitochondria. The most convincing results were obtained with GDP in the presence of exogenously added linoleic acid (LA), because this fatty acid is considered a strong positive effector of UCP isoforms. The stimulatory effect of GDP was revealed only under physiological-like conditions, i.e. those favoring oxidative phosphorylation (absence of carboxyatractyloside and oligomycin, inhibitors of adenine nucleotide translocase and F_OF_1-ATP synthase, respectively) and in the presence of ATP. As an explanation of the GDP-sustained stimulatory effect we propose the involvement of GDP in oxidative phosphorylation (OXPHOS) process because the GDP effect was fully sensitive to oligomycin, carboxyatractyloside or lack of inorganic phosphate in the incubation medium. OXPHOS effect in the presence of GDP could be a result of GDP OXPHOS taking into account: (i) carrier-dependent GDP import to the mitochondrial matrix and (ii) F_OF_1-ATP synthase properties, i.e. binding and phosphorylation of GDP. Alternatively, GDP could indirectly induce OXPHOS thanks to the activity of mitochondrial nucleoside diphosphate kinase (NDPK), an enzyme catalyzing the transfer of a γ-phosphate group from ATP to nucleoside diphosphates, e.g., ATP + GDP → ADP + GTP. In such a scenario ADP induces OXPHOS and GDP serves only as an acceptor of phosphate group derived from ATP. However, it must be kept in mind that GDP acts both as a substrate and an inhibitor of NDPK, causing complete inhibition of NDPK when reaching 0.6 mM concentration. Finally, we suggest the usage of GTP instead of GDP as a diagnostic inhibitor of UCP-mediated proton leak because under our physiological-like conditions GTP has never been stimulatory and gave expected recoupling effect, i.e. inhibition of LA-induced stimulation of respiratory rate accompanied by the restoration of LA-induced decrease of membrane potential.

Acknowledgements

This work was supported by grants of the Polish Ministry of Science and Higher Education (NN 301 636440 and Iuventus Plus program 2013-2015 IP2012 059172) and partially by European Union with resources from the European Regional Development Fund under the Innovative Economy Programme (POIG.01.01.02-00-069/09).

P14.17

Effect of NS1619 on mitochondria in EA.hy 926 endothelial cells

A. Łukasiak[1,2], A. Skup[2], A. Szewczyk[2], A. Wrzosek[2]

[1]Department of Biophysics, Warsaw University of Life Sciences SGGW, Warsaw, Poland; [2]Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, Warsaw, Poland
e-mail: Agnieszka Łukasiak <agnieszka_lukasiak@sggw.pl>

NS1619, a large conductance potassium (BK_{Ca}) channels opener is well known for its protective effect against ischemia-reperfusion injury, however, the exact mode of its action still seems unclear. The aim of this study was to determine the effect of NS1619 on endothelium.
As a model the endothelial cell line, EA.hy 926, was used. Changes in calcium concentration and mitochondrial membrane potential were measured using FURA-2AM and JC-1 fluorescent probes, respectively. Oxygen consumption was assessed with Clark-type oxygen electrode (Oroboros Instruments). ATP-synthase activity was determined in submitochondrial particles and solubilized mitochondria, using coupled enzyme reaction system method.
NS1619 caused dose-dependent increase in intracellular calcium ions $[Ca^{2+}]_i$ concentration in the EA.hy 926 cells. The $[Ca^{2+}]_i$ increase is related to the release of Ca^{2+} from endoplasmic reticulum. Additionally it was shown that NS1619 has rotenone-like properties and inhibited oxygen consumption rate in EA.hy 926 cells. However, when applied in the presence of oligomycin, NS1619 increased the oxygen consumption. Along with respiration, mitochondrial potential was measured. NS1619 decreased mitochondrial membrane potential in EA.hy 926 cells mitochondria. The result was different to rotenone which decreased mitochondrial membrane potential only when applied with oligomycin. The results indicate that NS1619 inhibits ATP-synthase in similar way to oligomycin. It was shown that in solubilized mitochondria, when the regulatory F_0 subunit of ATP-synthase is inactive, NS1619 and oligomycin had no effect on the synthase. However, in submitochondrial particles with intact complex of ATP-synthase, NS1619 inhibited the synthase in dose dependent manner.
In conclusion, NS1619 has pleiotropic actions on EA.hy 926 cells acting not only as a opener of BK_{Ca} channel opener, but it also has an effect on endoplasmic reticulum $[Ca^{2+}]_i$ storage, and mitochondrial ATP-synthase.

Acknowledgments

This study was supported by European Union from the resources of the European Regional Development Fund under the Innovative Economy Pro- gramme (grant coordinated by JCET-UJ, No POIG.01.01.02-00-069/09).