Session 19: Bioenergetics

Lectures

L19.1

Viral ion channels: small, active and informative

M. Kithil[1], A. Engel[1], B. Hertel, A. Moroni[2], G. Thiel[1]

[1]Membrane Biophysics, Department of Biology, Technische Universität Darmstadt; [2]Department of Biology, Università degli Studi di Milano, Via Celoria 26, 20133 Milano, Italy
Gerhard Thiel <thiel@bio.tu-darmstadt.de>

It is well established that proteins in the plasma membrane are synthesized in a co-translational manner while those in the membrane of mitochondria are sorted post-translationally to their site of activity. Considering these two very different pathways of protein synthesis it is interesting to realize that mitochondrial membranes contain a number of proteins, which resemble in structure and/or function those in the plasma membrane; among these are also several different types of K^+ channels. In order to understand the mechanism, which is responsible for a sorting of two similar channel proteins to two different destinations, we employ small viral K^+ channels. These proteins represent the pore domains of complex K^+ channels; one of these channels is sorted in mammalian cells into the inner membrane of the mitochondria, while the other channel arrives in the plasma membrane *via* the secretory pathway. We will present data, which indicate that the decision for the sorting of the channels is taken at the level of the nascent protein on the ribosome. A competition between different chaperons at the ribosome exit tunnel for the nascent protein determines the further sorting pathway. Binding of the nascent protein to these chaperons is determined by structural information on the n- and c-terminus of the channel protein.

L19.2

Translational and proteolytic control of plant mitochondrial biogenesis and function

Hanna Janska

University of Wroclaw, Faculty of Biotechnology, Molecular Biology of the Cell Department, Wrocław, Poland
Hanna Janska <hanna.janska@uwr.edu.pl>

Protein synthesis and protein degradation are two competing processes that determine the composition and quality of proteome. The key players in these processes are ribosomes and proteases, respectively. A study will be presented on the regulatory function of mitoribosomes and intramitochondrial proteolysis in biogenesis and the function of plant mitochondria.
Historically, ribosomes were considered to have a constitutive rather than a regulatory function. We have demonstrated that ribosomal-mediated control of translation exists in Arabidopsis mitochondria. Artificially altered mitoribosomes translate two subset of mRNA, those encoding components of oxidative phosphorylation complexes (OXPHOS) and mitoribosomal proteins, with altered efficiency.Ongoing experiments using ribosomal profiling technique are investigating the mechanism by which altered population of mitoribosomes leads to downregulation of OXPHOS subunits synthesis and upregulation of ribosomal proteins translation.
Intramitochondrial proteolysis regulates several mitochondrial functions through proteolytic processing or degradation. The results of our research will be presented in the context of the role of FTSH4, one of the mitochondrial inner membrane-embedded ATP-dependent metalloproteases in Arabidopsis, for functionality of the OXPHOS system, mitochondrial morphology and prevention of oxidative stress. The reduced cardiolipin content in mitochondria lacking FTSH4 is associated with perturbations within the OXPHOS complexes generating more reactive oxygen species, less ATP and with deregulation of mitochondrial dynamics causing in consequence the accumulation of oxidative damage. We postulate that the FTSH4 protease suppresses oxidative damage in plant mitochondria indirect by control of the cardiolipin abundance.

L19.3

Molecular mechanisms governing energy flows in the photosynthetic apparatus of plants

Wiesław I. Gruszecki, Ewa Janik, Joanna Bednarska, Rafał Luchowski, Monika Zubik, Karol Sowiński, Wojciech Grudziński, Renata Welc, Emilia Reszczyńska

Department of Biophysics, Institute of Physics, Maria Curie-Skłodowska University, Lublin, Poland
Wieslaw Gruszecki <wieslaw.gruszecki@umcs.pl>

Life on the Earth is powered by energy of sunlight but conversion of energy of electromagnetic radiation to the forms that can be utilized directly to drive biochemical reactions is possible owing to photosynthesis. On the other hand, light can also be toxic to photosynthesizing organisms, due to photo-oxidative damage. Plants have developed several strategies, during the millions of years of the biological evolution, to adapt to changing light intensity. Regulatory processes that underlie such an adaptation, operate at the level of entire organisms and at the cellular level, as well as at the molecular level of single, functional pigment-protein complexes. Studies of the largest photosynthetic pigment-protein antenna complex of plants, LHCII, reveal that the protein is involved both in light harvesting and excitation energy transfer to the reaction centers, under low light conditions, and in quenching of excessive excitations under strong illumination. The results of the recent studies carried out in our laboratory show that one of the central molecular mechanisms that trigger a physiological activity of LHCII, between light harvesting and photoprotection, is a trimer-to-monomer transition of the complex. Interestingly, this mechanism appears to be tightly related to operation of the xanthophyll cycle in the photosynthetic apparatus of plants. The xanthophyll cycle consist in a light-dependent deepoxidation of violaxanthin, leading to accumulation of zeaxanthin. According to the results of our studies, violaxanthin is involved in stabilization of supramolecular structures of LHCII, characterized by efficient excitation energy transfer. Destabilization of such structures, accompanying violaxanthin deepoxidation, is associated with formation of aggregated forms of the complex, characterized by high rate of protective energy dissipation. All the recent findings can be integrated within the model showing how molecular reorganization of the antenna complex LHCII governs energy flows in the photosynthetic apparatus of plants.

L19.4

Oxidative stress and mitochondrial abnormalities in skin fibroblasts derived from patients with the defects of the mitochondrial respiratory chain

Mariusz R. Wieckowski

Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw, Poland
Mariusz Wieckowski <m.wieckowski@nencki.gov.pl>

As the centres of oxidative metabolism, mitochondria are considered as the powerhouse of the cell and are involved in several other critical metabolic processes. For this reason and many others, defects in mitochondrial function are often associated with pathological states and can lead to various diseases. "Energetic catastrophe" is considered to be a major cause of pathogenesis of mitochondrial disorders and the clinical phenotypes observed in patients. Recently, oxidative stress is often considered not only to be a consequence of mitochondrial dysfunction but also a primary cause of mitochondrial disorders. The alteration in the mitochondrial respiratory chain is a cause of increased reactive oxygen species (ROS) production in mitochondria. Our studies addresses mitochondrial bioenergetics and oxidative stress to elucidate how these parameters participate in the pathogenesis of mitochondrial disorders. Complex characterization of mitochondrial respiratory chain function and the oxidative stress parameters, that are responsible for or involved in mitochondrial defect-mediated cellular dysfunction, may help us to characterize the impact of mitochondrial dysfunction on intracellular oxidative stress. Anomalies in the bioenergetic parameters, modification of the antioxidant enzymes levels as well as enhancement of intracellular ROS confirmed the occurrence of the oxidative stress in the studied fibroblasts. Principal component analysis showed, that individual defects have been grouped in the separated clusters. This indicates that mitochondrial defects can be characterized by a unique profile of cellular bioenergetic parameters and ROS homeostasis. We believe that such comprehensive analysis will suggest potential therapeutic strategies in which mitochondrial physiology or ROS production is modulated to alleviate the consequences of mitochondrial diseases.

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Oral presentations

O19.1

Endothelial mitochondria and elevated level of free fatty acids

Izabela Broniarek, Agnieszka Kozieł, Wiesława Jarmuszkiewicz

Department of Bioenergetics, Adam Mickiewicz University, Poznań, Poland
Izabela Broniarek <is18816@amu.edu.pl>

A chronic elevation of circulating FFAs is observed in obesity and diabetes type 2. Endothelial cells have permanent contact with blood and transported compounds, including free fatty acids (FFAs).The goal of this study was to assess the influence of exposure to elevated FFA level on the aerobic metabolism of endothelial cells. A human endothelial cell line EA.hy926 was chronically exposed to elevated level of palmitic acid (tested range 100-150 uM). We examined changes in respiratory functions of endothelial cells and endothelial mitochondria measuring: oxygen uptake with different respiratory substrates; total and mitochondrial reactive oxygen species (ROS) formation, expression level of uncoupling protein 2, superoxide dismutase 2 and marker proteins of glycolysis, anaerobic metabolism, β-oxidation, tricarboxylic acid cycle and mitochondrial respiratory chain. The exposure to high palmitic acid levels induces a shift in endothelial aerobic metabolism towards the oxidation of fatty acids. Increased levels of palmitic acid caused impairment and uncoupling of the mitochondrial oxidative phosphorylation system. 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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O19.2

Diverse functional responses of plant mitochondria in stress and recovery

Michal Rurek[1], Andrzej Woyda-Ploszczyca[2], Wieslawa Jarmuszkiewicz[2]

[1]Institute of Molecular Biology and Biotechnology, Department of Molecular and Cellular Biology, Faculty of Biology, Adam Mickiewicz University in Poznań, Poznań, Poland; [2]Institute of Molecular Biology and Biotechnology, Department of Bioenergetics, Faculty of Biology, Adam Mickiewicz University in Poznań, Poznań, Poland
Michal Rurek <rurek@amu.edu.pl>

Complex approaches to elucidate the biological relevance of plant mitochondrial responses to temperature stress and stress recovery are still limited. We investigated the functional aspects of the cauliflower curd mitochondrial biogenesis under cold, heat and after the subsequent stress recovery.

Activities of OXPHOS complexes and the ATP synthesis yield were affected in heat stress, which resulted in lowered OXPHOS efficiency likely due to the upregulation of AOX activity. Heat also increased the level of AOX protein and mRNAs for selected AOX isoforms while heat recovery reversed both AOX level and activity. In heat and heat recovery structural disintegration of matrix complexes and ATP synthase were observed. Such effects resulted partially from the lack of coordination between accumulation of transcripts and *de novo* synthesized complex subunits. Moreover, in heat recovery adaptive over-accumulation of some AOX messengers was evident.

Cold stress led to a decrease in complex II, complex IV and AOX activity (and AOX protein abundance); however, OXPHOS efficiency was almost unaffected. On the contrary, it was significantly lowered in cold recovery, due to the increased activity of rotenone-insensitive internal NADH dehydrogenase. Overall, activities of OXPHOS complexes were particularly affected after cold and heat recovery, where alterations in mitochondrial morphology were also evident, including programmed cell death in apical layer of cauliflower curds.

We conclude that cauliflower mitochondria respond to distinct temperature treatments in contrasting modes at functional level. Alterations in AOX activity under particular stress conditions could be controlled independent of protein abundance, e.g., by the regulation of transcript pool accessible for protein synthesis from diverse AOX genes. Notably, we show that plant AOX and rotenone-insensitive internal NADH dehydrogenase were reciprocally regulated in temperature stress.

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O19.3

The impact of human VDAC isoforms on cell viability of the yeast *Saccharomyces cerevisiae* Huntington's disease model

Daria Grobys, Andonis Karachitos, Hanna Kmita

Adam Mickiewicz University in Poznan, Institute of Molecular Biology and Biotechnology, Laboratory of Bioenergetics, Poznań, Poland
Daria Grobys <grobysdaria@gmail.com>

Huntington disease (HD) is an autosomal-dominant neurodegenerative disorder characterized by a loss of neurons, mainly in striatum and cerebral cortex. The disease is caused by CAG trinucleotide repeat expansion in exon 1 of *HTT* gene encoding huntingtin (Htt). The repeats number higher than 35 results in an abnormally long polyglutamine tract in Htt N terminus that gives rise to its mutant form (mHtt). It is now obvious that mitochondria play a vital role in HD pathogenesis but the underlying mechanism is still elusive. Moreover, the involvement of Htt in mitochondria functioning is still unclear although it is becoming increasingly apparent that mHtt can impair mitochondrial function. Voltage-Dependent Anion-selective Channel (VDAC) appears to be a possible target of Htt and mHtt as the channel contributes to mitochondrial proceses directly or by interaction with involved proteins. Importantly, three different VDAC isoforms (VDAC1, VDAC2, and VDAC3) have been characterized in vertebrate mitochondria including human ones but their specific roles are still not explained. To investigate the role of human VDAC isoforms for cell viability in HD pathogenesis, we applied the yeast *Saccharomyces cerevisiae* HD model based on galactose induced expression of *HTT* gene exon 1 containing 25 or 103 repeats of glutamine codon that results in synthesis of Htt and mHtt, respectively that can be monitored due to GFP labeling. The obtained results indicate that human VDAC isoforms contribute differently to cell viability under the applied conditions of direct and indirect effects of Htt and mHtt expression.

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O19.4

Mechanosensitivity of mitochondrial large-conductance Ca^{2+}-activated K^+ channel

Agnieszka Kiełbasa, Piotr Koprowski, Bogusz Kulawiak, Adam Szewczyk

Laboratory of Intracellular Ion Channels, Department of Biochemistry, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw,Poland
Adam Szewczyk <a.szewczyk@nencki.gov.pl>

Large-conductance Ca^{2+}-activated K^+ channels are regulated by voltage and agents such as divalent cations or fatty acids. They fulfill number of functions in cell physiology and are found in different cellular compartments including plasma membrane, nucleus and mitochondria. Mitochondrial large-conductance Ca^{2+}-activated K^+ channel (mitoBK_{Ca}) resides in the inner mitochondrial membrane (IMM) and plays an important role in cytoprotection. It is formed by DEC splice variant of *KCNMA1* gene. Here we show that mitoBK_{Ca} is also sensitive to stretching of IMM suggesting its physiological role during mitochondria swelling. We have tested by usage of patch-clamp technique the relation between mechanosensitivity of mitoBK_{Ca} channels and impact of small molecule modulators such as potassium channel openers and carbon monoxide on its activity.

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Posters

P19.1

Age-related structural changes in the liver of old mice with SIRT1 overexpression in conjunction with NMN supplementation

Peter Cao[1,2], Victoria Cogger[1,2], Lindsay Wu[4], David Le Couteur[1,2,3]

[1]ANZAC Research Institute, Concord, NSW, Australia; [2]The University of Sydney, Camperdown, NSW, Australia; [3]Ageing and Alzheimers Institute, Concord Hospital, Concord, NSW, Australia; [4]School of Medical Sciences, The University of New South Wales, Randwick, NSW, Australia
Peter Cao <ptrcao@gmail.com>

Aging is associated with structural changes in the liver, including a reduction in hepatocyte mitochondrial number density and liver sinusoidal porosity. Histologically-verifiable changes include lipid and collagen accumulation, an increase in α-smooth muscle actin (α-SMA) – reflecting the activation of hepatic stellate cells – and the elevation of von Willebrand factor (vWf) levels. Sirtuin 1 (SIRT1), an NAD^+-dependent deacetylase, is renowned for its range of anti-aging actions. This study was interested to see if SIRT1's anti-ageing effects would extend to the aforementioned liver ageing characteristics.

Here we have evaluated the effect of whole-body SIRT1 overexpression on the livers of young (3-5 month) and old (13–20 month) SIRT1 transgenic mice and their wildtype controls. In addition, due to concerns about diminished NAD^+ levels with age, some old animals have been supplemented with the NAD^+ precursor, nicotinamide mononucleotide (NMN).

Transmission electron microscopy determination of liver mitochondrial numerical density (mitochondrial particles per cytosolic μm^2) showed that there was no change with age, nor a treatment effect of SIRT1 overexpression or NMN supplementation.

Analysis of the liver blood vessels with scanning electron microscopy revealed that SIRT1 overexpression was able to increase sinusoidal porosity (%) in older but not younger mice ($F(1.8) = 14.9$, $p<0.01$). However, since SIRT1 overexpression in old mice did not merely restore porosity to younger levels, but doubled porosity relative to younger mice, an anomaly emerged in which the young mice were unable to benefit from SIRT1 overexpression to the same extent as their older counterparts.

Histological examination of the mice's livers showed that age was associated with an increase in lipid and collagen levels, as expected. However, there was no effect of age on the presence of activated stellate cells or levels of vWf. Not so well understood was the finding that mice that were treated with NMN were 0.11 times less likely to have elevated α-SMA levels, yet a combination of SIRT1 overexpression and NMN supplementation led to a 20.43 times greater likelihood of elevated α-SMA levels.

The lack of canonical ageing-associated changes in a number of regards raises the question of whether the older mice were sufficiently old for such effects to emerge.

P19.2

Mitochondrial uncoupling protein 2 in endothelial cells

Izabela Broniarek, Agnieszka Koziel, Wieslawa Jarmuszkiewicz

Department of Bioenergetics, Adam Mickiewicz University, Poznań, Poland
Wiesława Jarmuszkiewicz <wiesiaj@amu.edu.pl>

Uncoupling proteins (UCPs) mediate free fatty acid-activated, purine nucleotide-inhibited proton conductance through the inner mitochondrial membrane. Products of lipid peroxidation can activate UCPs and promote feedback down-regulation of mitochondrial reactive oxygen species (ROS) formation. UCP2 works as a regulator of mitochondrial ROS generation and can antagonise oxidative stress-induced endothelial dysfunction. Our studies revealed the important role of UCP2 in hyperglycaemia- or hyperlipidemia-induced modifications of mitochondrial function in endothelial cells. We demonstrated that primarily glycolytic endothelial cells possess mitochondria with UCP2 as a functional energy-dissipating system. In phosphorylating endothelial mitochondria, UCP activity efficiently diverts energy from oxidative phosphorylation (decreases ATP synthesis) by dissipating the proton electrochemical gradient. In non-phosphorylating endothelial mitochondria, the UCP-mediated uncoupling is revealed by stimulation of the respiratory rate and a decrease in membrane potential and the ubiquinone reduction level. Thus, by dissipation of the proton electrochemical gradient, UCP activity allows an increase in the electron flux at the expense of ubiquinol, which lies at the basis of UCP antioxidant function (i.e., attenuation of potentially damaging mitochondrial ROS production). UCP2 gene silencing led to an increased inflammatory activation that was accompanied by the reduced endothelial cell viability and resistance to oxidative stress. Under phosphorylating conditions, the increased UCP2 activity in the mitochondria isolated from endothelial cells exposed to high glucose concentrations or high palmitate concentrations led to a significantly higher reduction in the oxidative phosphorylation yield (ATP synthesis). Moreover, a more pronounced hyperglycaemia- or hyperlipidemia-induced control of the respiratory rate, membrane potential and ROS production by UCP activity in non-phosphorylating endothelial mitochondria was observed. In endothelial cells, the hyperglycaemia- or hyperlipidemia-induced upregulation of UCP2 reduced mitochondrial membrane potential and decreased the production of mitochondrial ROS, which promotes increased stress resistance and protection against acute oxidative stress.

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P19.3

VDAC mediates cell viability: a case report of PC12 model of Huntington's disease

Andonis Karachitos, Daria Grobys, Klaudia Kulczyńska, Adrian Sobisiak, Hanna Kmita

Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University in Poznań, Poznań, Poland
Hanna Kmita <kmita@amu.edu.pl>

It is becoming increasingly apparent that mitochondria dysfunction plays an important role in pathogenesis of Huntington's disease (HD) but the underlying mechanism is still elusive. Thus, there is a still need for further studies concerning the upstream events in mitochondria dysfunction that could contribute to cell death observed in HD. Taking into account the fundamental role of the voltage-dependent anion-selective channel (VDAC) in mitochondria functioning it is reasonable to consider the channel as a crucial element in HD etiology. Therefore, we applied PC12 inducible cell culture model of HD to determine the relationship between the effect of expression of wild type and mutant huntingtin (Htt and mHtt, respectively) on cell survival and mitochondria functioning in intact cells under conditions of undergoing cell divisions. As a clear difference between the phenomena occurred after 48 h of Htt or mHtt expression we decided to estimate the effect of Htt and mHtt expression lasted for 48 h on VDAC functioning. The presented data indicate that the first measurable mitochondrial symptom of mHtt expression in inducible HD PC12 cell model is change of basal respiration and subsequent changes of cell viability coexisted with changes of the status of mitochondrial coupling in intact cells accompanied by changes of reconstituted VDAC, being probably mainly VDAC1, properties. Therefore the data appear to be important for better understanding of cytotoxicity as well as cytoprotection mechanisms of potential clinical application.

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P19.4

Aerobic metabolism of human endothelial cells under hypoxic conditions

Agnieszka Koziel, Izabela Broniarek, Wieslawa Jarmuszkiewicz

Department of Bioenergetics, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland
Agnieszka Koziel <koziel@amu.edu.pl>

We examined the influence of hypoxic conditions on the aerobic metabolism of endothelial cells and isolated mitochondria. Human umbilical vein endothelial cells (EA.hy926) were cultured under two different oxygen concentrations, 21% (normoxia) and 3% (hypoxia). Hypoxia lead to accumulation of hypoxia-inducible factor 1 (alpha subunit, HIF1a), which is response marker to hypoxia.

We measured oxygen uptake by EA.hy926 cells with different respiratory substrates: pyruvate, glucose, glutamine and palmitate. Respiratory response to hypoxia was observed in cells grown for at least 6 days or longer. We determined the activity of citrate synthase (CS), cytochrome c oxidase and lactate dehydrogenase (LDH). Furthermore, we determined expression level of hexokinase I, LDH, and CS. Significantly higher activity of LDH was accompanied by greater expression level of this protein in hypoxic endothelial cells.

We measured mitochondrial activity of isolated mitochondria (oxygen uptake and membrane potential) with following substrates: malate, glutamate, pyruvate, succinate, glycerol-3-phosphate, and palmitoylocarnityne. We determined expression level of complexes of mitochondrial respiratory chain, ATP synthase, pyruvate dehydrogenase, acyl-coenzyme A dehydrogenase. We measured influence of hypoxia on mitochondrial and non-mitochondrial ROS production. ROS generation was significantly higher in hypoxic endothelial cells.

Endothelial mitochondria may play a central role in development of many cardiovascular diseases. The oxidative stress induced by hypoxia is an important aspect of these diseases. Endothelial mitochondria may play an important role in hypoxic sensing.

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P19.5

Single channel properties and topology of the ROMK2 – pore forming unit of the mitoK$_{ATP}$ channel

Michal Laskowski, Bogusz Kulawiak, Adam Szewczyk

Nencki Institute of Experimental Biology, Laboratory of Intracellular Ion Channels, Warsaw, Poland
Adam Szewczyk <a.szewczyk@nencki.gov.pl>

Ischemic heart disease remains one of the leading cause of death in the developed world, and endogenous mechanisms to reduce cardiac ischemic damage are being actively investigated. Activation of the mitochondrial ATP-sensitive potassium (mitoK$_{ATP}$) channel has been implicated in the mechanism of cardiac ischemic preconditioning. Recent studies suggest that isoform of the renal outer medullary potassium channel (ROMK2) is a pore forming component of the mitoK$_{ATP}$ channel [1]. Our goal was to investigate single channel properties of ROMK2. Patch-clamp technique measurements, on isolated mitoplasts from H9c2 cells with overexpression of the ROMK2, were applied to determine electrophysiological properties of the channel. To identify pharmacological properties of mitoK$_{ATP}$ channel formed by ROMK2 protein, channel activity inhibitors and potassium channel openers were used. Our results confirm that ROMK2 channel have typical biophysical properties of the mitoK$_{ATP}$ channel. Using immunoblotting technique we confirm presence of channel in the mitochondrial inner membrane. We also determine the topology of the channel. Electrophysiological and biochemical confirmation that ROMK2 is pore forming unit of mitoK$_{ATP}$ channel may lead to better understanding it's cytoprotective role and may contribute to development of novel therapeutical tools.

P19.6

Is mitochondrial NDPK involved in the regulation of the GDP inhibitory effect on uncoupling proteins?

Andrzej M. Woyda-Ploszczyca, Wieslawa Jarmuszkiewicz

Adam Mickiewicz University in Poznan, Faculty of Biology, Department of Bioenergetics, Poznań, Poland
Andrzej Woyda-Płoszczyca <awoy@amu.edu.pl>

Purine nucleotide (PN) GDP is commonly used as a diagnostic inhibitor of mitochondrial uncoupling proteins (UCPs), the main catalyst of futile proton leak of proton electrochemical gradient generated by pumps of respiratory chain. However, GDP is also a substrate for many mitochondrial proteins such as mitochondrial nucleoside diphosphate kinase (mNDPK). This enzyme catalyzes the transfer of a γ-phosphate group from ATP (and other nucleoside triphosphates) to nucleoside diphosphates, e.g., ATP + GDP → ADP + GTP, to maintain relatively stable levels of PN pools. Our functional studies on isolated mitochondria of unicellular and multicellular eukaryotes have shown for the first time that mNDPK probably regulates the efficiency of GDP-dependent inhibition of UCPs. Namely, we postulate that mNDPK through transphosphorylation of GDP to ADP induces oxidative phosphorylation (OXPHOS) thus completely attenuates the GDP inhibitory effect on free fatty acids-stimulated UCP activity. Therefore, no OXPHOS-inducing GTP, instead of OXPHOS-inducing GDP, should be commonly used as the diagnostic inhibitor of UCP homologues. These observations change our basicknowledge of PN influence on UCPs thus the overall influence of PNs on energy transduction in mitochondria.

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