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„Mitochondria in the spotlight”

ABSTRACT BOOK

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Lectures and oral presentations

O.1 PPR proteins and the evolution of mitochondrial gene expression in yeasts

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Pentatricopeptide (PPR) motif proteins are the most numerous family of RNA binding factors, found in all Eukaryotic lineages. These nuclear-encoded proteins localize to mitochondria (and chloroplasts) and play diverse roles in post-transcriptional steps of organellar gene expression. They thus play a pivotal role in the co-evolution of host and endosymbiont shaping the eukaryotic genome landscape. They are particularly abundant in vascular plants, with hundreds of paralogous members in a typical genome. In spite of structural similarities, PPR proteins of plants and yeasts show interesting differences in their physiological roles and substrate recognition mechanisms. Yeast PPR proteins include general RNA binding factors, like Rmd9, as well as gene-specific regulatory proteins, like Aep2. A typical yeast genome encodes about a dozen of PPR proteins, and their complement reflects variation in the coding repertoire of the mitochondrial DNA. For example, in *Candida albicans* we identified four PPR proteins involved in the expression of mitochondrially encoded subunits of Complex I, that are absent from *S. cerevisiae*. Even when the PPR proteins and their mitochondrial targets are conserved between different yeast species, interspecies functional compatibility is usually quickly lost. A growing body of evidence indicates that nucleo-mitochondrial compatibility mediated by the interactions of PPR proteins with their target RNAs plays a major role in speciation in yeasts. As an example, a combination of biochemical approaches with comparative sequence analysis allowed us to identify the binding site of *S. cerevisiae* Dmr1 PPR protein, and show that it is involved in the evolution of nucleo-mitochondrial compatibility.

O.2 Structural basis for DNA replication and repair in human mitochondria

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In mammalian cells, genetic information is stored in two locations: in the nucleus and in mitochondria. DNA in mitochondria, just like in the nucleus must be faithfully copied and mistakes i.e. mutations due to exogenous and endogenous DNA damaging agents lead to formation of DNA lesions. Persistence of these DNA lesions may lead to genomic instability and human diseases like cardiovascular, skeletal muscular and neurological disorders, cancer as well as normal aging process. Although great progress towards understanding mitochondrial DNA metabolism has been made, relatively little is known about human mitochondrial DNA replication and DNA repair pathways. While there are a number of DNA repair enzymes shared by the nucleus and mitochondria, only human mitochondrial DNA polymerase γ (Polγ) and 5'-exo/endonuclease (EXOG) are mitochondria-specific and critical for both DNA replication and repair in human mitochondria. Interestingly, EXOG and Polγ, are proposed to complex with APE1 and Lig3 to form a large macromolecular machine called “mitochondrial repairsome”, proposed to coordinate DNA repair process in human mitochondria. We recently solved replicating, ternary structures of Polγ and illuminated the mechanism of allosteric regulation of this multi-subunit DNA polymerase. Concurrently, we solved several atomic resolution structures of EXOG and provided structural basis for its action in human mitochondrial DNA repair. Now, using a combination of structural biology (x-ray crystallography and electron microscopy), biochemistry, biophysics and cell biology we tackle the organization, structure and function of human mitochondrial repairosome.

O.3 Poly(A) polymerase primes degradosome-mediated decay of dsRNA in human mitochondria as revealed by RNAi screen

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Expression of the human mitochondrial genome due to transcription of both mtDNA strands is especially prone to produce dsRNA molecules. However, studies conducted so far focused mostly on dsRNA arising from nuclear transcription or exogenic viral sources. We have recently discovered that mitochondria are a major source of cellular dsRNA (Dhir *et al*., Nature 2018). Furthermore, we found that the level of mt-dsRNA is restricted by the mitochondrial degradosome, a complex of the ribonuclease PNPase and RNA helicase SUV3. Also, PNPase prevents dsRNA from leaking into the cytoplasm, where it induces a potent interferon response, which can contribute to human diseases.

In order to identify other factors involved in mt-dsRNA metabolism we performed an siRNA screening using a custom siRNA library. We screened 270 genes encoding proteins involved in different aspects of mitochondrial biology and observed several cases of up- and down-regulation of mt-dsRNA. We have discovered an interesting link between mitochondrial RNA processing enzymes and the mitochondrial degradosome.

One of the most prominent hits was a mitochondrial poly(A) polymerase, MTPAP. Its silencing led to robust accumulation of dsRNA comparable to inactivation of degradosome components. In vitro reconstitution of dsRNA decay reactions revealed that polyadenylation is a prerequisite of degradation of blunt mt-dsRNAs by the degradosome, which was further supported by in vivo experiments.

In conclusion, our study revealed the decay pathway of dsRNA in mitochondria and allowed us to identify several other factors involved in mitochondrial dsRNA metabolism.

O.4 Dedicated surveillance mechanism controls G-quadruplex forming non-coding RNAs in human mitochondria

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The GC skew in vertebrate mitochondrial genomes results in synthesis of RNAs that are prone to form G-quadruplexes (G4s). Such RNAs, although mostly non-coding, are transcribed at high rates and are degraded by an unknown mechanism. Here we describe a dedicated mechanism of degradation of G4-containing RNAs, which is based on cooperation between mitochondrial degradosome and quasi-RNA recognition motif (qRRM) protein GRSF1. This cooperation prevents accumulation of G4-containing transcripts in human mitochondria. In vitro reconstitution experiments show that GRSF1 promotes G4 melting that facilitates degradosome-mediated decay. Among degradosome and GRSF1 regulated transcripts we identified one that undergoes post-transcriptional modification. We show that GRSF1 proteins form a distinct qRRM group found only in vertebrates. The appearance of GRSF1 coincided with changes in the mitochondrial genome, which allows the emergence of G4-containing RNAs. We propose that GRSF1 appearance is an evolutionary adaptation enabling control of G4 RNA.

O.5 Pseudouridylation events in mitochondrial transcriptome

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Pseudouridylation is the most common RNA modification, yet little is known about the modified positions and their functional consequences. Until recently, due to technical limitations in pseudouridine detection, its role was thought to be primarily related to maintaining stability and structure of ribosomal and transfer RNA. Recently, a series of deep sequencing methods has been published that enable mapping Ψ positions across the entire transcriptome with single nucleotide resolution. These data have greatly expanded the catalogue of pseudouridylated transcripts including coding sequences of mRNA. Although the physiological significance of mRNA pseudouridylation remains still unclear it is suspected to be involved in response to cellular stressors given that the pseudouridine landscape changes substantially in different growth conditions. Such potential for a dynamic regulation of RNA function is especially interesting in case of human mitochondria where gene expression is mainly regulated post-transcriptionally. Interestingly, it was also found that mutation in PUS1 gene, which encodes pseudouridylate synthase that catalyzes the conversion of uridine to pseudouridine causes mitochondrial myopathy and sideroblastic anemia. Thus, PUS1 may play a yet important and unexplored role in human mitochondrial RNA metabolism.

In our project we aim at further investigation of mitochondrial epitranscriptome. To identify new pseudouridine positions and confirm previously described we will reanalyze recently published datasets from genome-wide studies of RNA pseudouridylation in humans that neglected mitochondrial transcripts. Next, we will examine whether detected modification depends on PUS1.

O.6 Mitoribosome profiling reveals molecular basis of selective translation in *rps10* mitochondria

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Heterogeneous population of mitoribosomes in *rps10 Arabidopsis thaliana* mutant alters translation in comparison to the wild-type plants (Kwasniak et al., 2013). The mitochondrial transcripts encoding OXPHOS proteins are less actively translated, whereas transcripts of ribosomal proteins are preferentially synthesized in *rps10*. To monitor the mitogenome-wide translation in *rps10* we performed mitoribosomal profiling (mtRibo-seq). This approach relies on deep sequencing of mitoribosome protected fragments (mtRPFs) reflecting the exact positions of ribosomes on transcript. mtRibo-seq was coupled with total mitochondrial RNA sequencing (mtRNA-seq).

Translational efficiency (TEs), which directly measures the efficiency of mRNA utilization, defined as the ratio of translational output to mRNA abundance, confirmed differential translation of OXPHOS and ribosomal mRNAs in *rps10* mitochondria compared to wild type. Interestingly, mitoribosome profiling reveals that a significant part of reads in *rps10,* but not in wild type was mapped outside of CDSs, to non-coding regions, both for mtRibo-seq and mtRNA-seq. Moreover, reads mapping on 3’ untranslated regions (3’UTR’s) clearly accumulate in mtRibo-seq compared to mtRNA-seq. A lack of three-nucleotide periodicity of 3’UTR reads indicate that they are not representing actively translated ribosomes. Surprisingly, we found that these reads coincide with sequences of small RNAs called cosRNAs (Ruwe et al., 2016), which likely originate *in vivo* from protection of RNA fragments against nuclease degradation by RNA binding proteins. In *rps10* the ratio of reads mapping on 3’UTRs to reads mapping on CDSs increases for OXPHOS and slightly decreases for ribosomal transcripts compared with the wild type. Thus, our study uncover a correlation between accumulation of reads, which resembles cosRNAs, mapping on 3’UTRs and reduced synthesis of OXPHOS proteins in *rps10*.

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O.7 Structural studies of the yeast mitochondrial RNA degradosome

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Degradation pathways play a key role in RNA metabolism, from the regulation of gene expression to the efficient removal of defective RNA molecules. The main RNA-degrading enzymes are processive exoribonucleases that together with other proteins can organize into macromolecular complexes gaining new functions. In yeast the main executor of mitochondrial RNA degradation is the mtEXO complex composed of Dss1 3′-to-5′ exoribonuclease and Suv3 helicase that act in concert and efficiently remove defective RNAs and excised introns. Crystal structure of Dss1 from *Candida glabrata* reveals it is a unique member of the RNB superfamily of ribonucleases with specialized domains responsible for interactions with Suv3 helicase. The arrangement of both subunits deciphered in the crystal structure of the complex enables the helicase motor to feed the 3′ end of the RNA into the catalytic channel of Dss1 for effective degradation. This co-operation of both helicase and nuclease activities within the complex is particularly important for degradation of structured RNAs which cannot be handled by Dss1 on its own and for which the unwinding activity of Suv3 is required [1].

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O.8 Potassium channels in cardiac mitochondria

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The increased flux of potassium ions into mitochondrial matrix through the mitochondrial potassium channels has been implicated in the mechanism of cytoprotection. However, details of this phenomenon remain unclear. The crucial role in observed cardioprotection play ATP-sensitive potassium channel (mitoK_{ATP}) and large conductance calcium regulated potassium channel (mitoBK_{Ca}). Recently, it has been suggested that the mitochondrial-targeted isoform of the renal outer medullary potassium channel (ROMK) protein create a pore forming subunit of the mitoK_{ATP} channel in the inner membrane of heart mitochondria.

Here, we focused on biophysical and pharmacological properties of potassium channels present in heart derived H9c2 cells. In our models we could detect channels corresponding to both mitoBK_{Ca} and mitoK_{ATP}. Detailed analysis of the mitoK_{ATP}-like activity using the mitoplast patch-clamping methods, revealed presence of a potassium channel with a mean conductance of 94 pS in symmetric 150/150 mM KCl. The activity of the channel was inhibited by ATP/Mg^{2+} and 5-hydroxydecanoic acid and partially inhibited by glibenclamide. The channel was activated by the mitoK_{ATP} channel opener diazoxide. The recorded activity was also blocked by Tertiapin Q, a known inhibitor of the ROMK-type channels. Additionally, our data suggest that both N- and C- terminal part of the channel are located in mitochondrial matrix. Based on above we conclude that in cardiac cells observed channel corresponding to mitoK_{ATP} is formed by ROMK protein.

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O.9 Regulation of mitochondrial potassium channels by cardioprotective flavonoids

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Flavonoids belong to a large group of polyphenolic compounds that are widely present in plants. Certain flavonoids, including luteolin, quercitin or cyanidin, have cytoprotective properties. Although the antioxidant effect has long been thought to be a crucial factor accounting for the cellular effects of flavonoids, mitochondrial channels recently emerged as targets for cytoprotective strategies. The aim of these studies was the characterization of interactions between cardioprotective flavonoids and the mitoBK$_{Ca}$ channel present in the inner mitochondrial membrane of endothelial cells.

In the current study, single channel activity of the mitoBK$_{Ca}$ channel was measured after patch-clamp of the mitoplasts isolated from endothelial cell line (EA.hy 926). We show detailed data describing regulation of the mitoBK$_{Ca}$ channel by the cardioprotective flavonoids (luteolin, quercitin and cyanidin). We have observed that opening probability of the channel increased from 0.15 in the control conditions (100 μM Ca^{2+}) to 0.26 after application of cyaniding in micromolar concentration range.

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O.10 *Saccharomyces cerevisiae* model sheds a new light on the activity of ADP/ATP carrier (AAC) – the importance of nucleoside diphosphate kinase (NDPK)

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In case of *S. cerevisiae* the AAC is considered the main catalyst of the futile (non-phosphorylating) proton leak across the inner mitochondrial membrane. The AAC-mediated H^+ leak is inhibited by both (i) carboxyatractyloside (CATR), a highly specific inhibitor of this carrier for adenine nucleotide (ADP and ATP) translocation, and (ii) GDP. Taking into account the toxicity of CATR for most organisms, this compound cannot function as the physiological inhibitor of AAC-sustained H^+ leak. The GDP metabolism in mitochondria may be in turn an obstacle for establishing this nucleotide as the native negative regulator of AAC-catalyzed H^+ leak. The NDPK transfers a γ-phosphate group from NTP to NDP, e.g., ATP + GDP → ADP + GTP. In mitochondria, this enzyme may be responsible for efficient transphosphorylation of GDP.

Mitochondria isolated from a wild type yeast strain and a mutant with disrupted gene for NDPK were used in studies. Results taken from simultaneous measurements of oxygen uptake (Clark-type electrode) and mitochondrial membrane electrical potential determined with the tetraphenylphosphonium cation (TPP^+)-selective electrode allowed to put forward a new hypothesis.

In contrast to earlier studies, the addition of GDP (1 mM) stimulated the state 4 (non-phosphorylating respiration)-state 3 (phosphorylating respiration) transition, but only in mitochondria isolated from wild type yeast strain. Therefore, NDPK seems to be the major consumer of GDP pool in the intermembrane space of mitochondria, thus the GDP inhibitory effect on AAC is rather negligible.

This work was supported by a grant from the National Science Centre, Poland (2015/19/D/NZ3/00087).

O.11 PGC-1α controls the interplay between senescence, autophagy and mitochondrial quality control in the aging retina

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Cell senescence is a major contributor to the loss of fitness associated with aging and age-related diseases. Senescent cells produce more reactive oxygen species and have damaged mitochondria and genes crucial for mitochondrial homeostasis. Senescence results in dysregulated mitophagy that drives senescence associated mitochondrial dysfunction, a significant cause of accelerated aging. Central retinal pigment epithelium (RPE), a critical region in many retinal diseases, contains cells which are quiescent due to spatial constraints and when damaged, they can be replaced by their proliferating counterparts at RPE periphery. If most of RPE cells, including periphery, are senescent, this mechanism can fail leading to retina diseases. Therefore, senescence and not programmed cell death is crucial for RPE degeneration and we have built a model of age-related macular degeneration (AMD) with senescence, autophagy and mitochondrial quality control as critical elements. In a recently discovered senescence regulatory pathway the activation of the ATM, Akt and mTOR phosphorylation cascades downstream of DNA damage triggers PGC-1α-dependent mitochondrial biogenesis. We use ARPE-19 cells, RPE cells obtained from mice at two different ages with the knockout in the *PGC-1α* gene and RPE cells obtained from human induced pluripotent stem cells in which *PGC-1α* expression is regulated by the CRISPR/Cas9 technology. In the results obtained so far we showed that inhibition of autophagy in ARPE-19 cells resulted in fragmentation of the mitochondrial network, RPE cells of PGC-1α KO mice showed a changed expression of autophagy-related genes and an increased number of damaged mitochondria and these changes potentiated with age.

O.12 Mitochondria, protein homeostasis and aging

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Among several cellular quality control systems, that are responsible for keeping mitochondria healthy, the least understood is recently discovered Unfolded Protein Response activated by mistargeted proteins (UPRam). UPRam is being activated due to defects in the transport of the mitochondrial protein. It acts through proteasome, a large complex responsible for protein degradation in the cell, to reduce the accumulation of the mitochondrial precursor proteins in the cytosol and to reestablish proteins homeostasis. While first described in yeasts, UPRam presence in higher eukaryotes was thus far not explored.

In our work, we investigated the role of UPRam in the nematode *Caenorhabditis elegans*. Our results show that mild mitochondrial stress caused by the inhibition of mitochondrial protein import is beneficial for the whole organism and it prolongs *C. elegans* lifespan. This phenotype depends both on the activity of the proteasome and on the Mitochondrial Unfolded Protein Response (UPRmt). Furthermore, we could observe that there is a spatial relationship between mitochondria and proteasome, manifested by the presence of proteasome very close to the mitochondria surface. Taken together, the results of our project show the tight cross-talk between the mitochondria and the proteasome, and its importance in the mitochondrial health regulation.

O.13 Mitochondrial dysfunction in Non-Alcoholic Fatty Liver Disease

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Non-Alcoholic Fatty Liver Disease (NAFLD) is a common disease in Western society and ranges from steatosis to steatohepatitis and to end-stage liver disease. Sedentary lifestyles, dietary changes, epidemic obesity and type 2 diabetes further contribute to the worldwide increase in NAFLD, which currently affects 25% of the worldwide population. The molecular mechanisms that cause the progression of steatosis to severe liver damage are not fully understood. One suggested mechanism involves the oxidation of biomolecules by mitochondrial ROS which initiates a vicious cycle of exacerbated mitochondrial dysfunction and increased hepatocellular oxidative damage. Steatosis can progress to inflammatory Non-Alcoholic SteatoHepatitis (NASH), fibrosis, cirrhosis and hepatocellular carcinoma, ultimately culminating in liver failure.

It has been documented, that hepatic mitochondria are structurally and molecularly altered in NAFLD. As the cell powerhouse, a decline in mitochondrial function, concomitant with structural and molecular alterations, may provoke metabolic disturbances and may potentially contribute to NAFLD progression. However, the sequence of events and signaling pathways that link mitochondrial remodeling and dysfunction to stages of NAFLD progression remain unclear.

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O.14 Studies of mitochondrial mutations in complex III using photosynthetic bacterial model

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Mitochondrial complex III is one of the key enzymes of the respiratory chain. It catalyses electron transfer from quinone to cytochrome *c* pools, what is associated with generation of proton motive force, utilized for ATP production. Its catalytic core consists of cytochrome *b*, cytochrome c_1, and iron-sulfur protein (ISP), which performs movement during the enzymatic cycle. Mutations in mtDNA-encoded cytochrome *b* cause various mitochondrial diseases, but studying their molecular effects in eukaryotic organisms is difficult. Therefore, we use a model system based on photosynthetic purple bacteria *Rhodobacter capsulatus* that allows straightforward mutagenesis and various analyses of the bacterial homologue of the complex III. Taking advantage of the paramagnetic properties of metal cofactors embedded in the complex we used electron paramagnetic resonance to discover that two mutations located at the cytochrome *b* – ISP interface, G167P and G332D, affect the movement of the ISP. The magnitude of the shift of ISP from the quinol oxidation catalytic site is correlated with the levels of superoxide produced by the enzyme. We used the same approach to decipher the molecular effects of the D254N mutation that appeared spontaneously in the *Bcs1l* mutant mouse colony, shortening lifespan and affecting mice metabolism. The equivalent of D254N in bacteria affected the ISP movement but without change in the superoxide production. As the nuclear *Bcs1l* mutation causes the partial loss of ISP, our findings represent a unique case of mitonuclear epistasis. We anticipated that these mouse and bacterial models will be useful in the future studies of complex III.

O.15 Anti-platelet effects of carbon monoxide-releasing molecules

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Endogenous carbon monoxide (CO) produced by haem oxygenases plays an important role in the cardiovascular system including the prevention of excessive activation of platelets and inhibition thrombotic processes. In turn, CO-releasing molecules (CO-RMs) afford anti-platelet and anti-thrombotic effects. In some cells, pharmacological activity of CO-RMs share a similar mechanism of action as NO-donors. Some time ago, we demonstrated that in contrast to anti-platelet effects of nitric oxide (NO), the mechanism of the antiplatelet activity of CO-RMs did not involve the stimulation of soluble guanylate cyclase *[Chlopicki S et al, Cardiovasc Res. 2006;71(2):393-401]*. We also demonstrated that anti-platelet effects of CO-RMs displayed a distinct dependence on the kinetics of CO-release as compared with NO-donors [*Chlopicki S et al, NS Arch Pharmacol. 2012;385(6):641-50*]. Indeed, CORM-A1, a prototypic CO-RM slowly releasing CO, was shown to afford anti-platelet and anti-thrombotic activities in vivo without any hypotensive effect, while CORM-3, which releases CO instantly, displayed both anti-thrombotic and hypotensive effects *[Kramkowski K et al, ATVB 2012;32(9):2149-57]*. Recently, a new class of cis-rhenium(ii)-dicarbonyl-vitamin B12 complexes (B12-ReCORMs) with tuneable CO releasing properties were synthetized, and couple of them displayed anti-platelet activity similar or even slightly more pronounced than CORM-A1, confirming the significant anti-platelet effect of CO-RMs *[Prieto et al, Chem Commun (Camb). 2017 22;53(51):6840-6843]*. Although previous studies repeatedly demonstrated that CO-RMs inhibit platelet aggregation in *in vivo* and *in vitro* models, the mechanism of the antiplatelet activity of CO-RMs has not been elucidated. Results will be presented to suggest that the modulation of platelet bioenergetics may contribute to the anti-platelet effects of CORM-A1.

O.16 The significance of plant mitochondria during ammonium nutrition

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Nitrogen assimilation by plants requires a high load of energy and reductants. Therefore it may be expected that the use of the reduced form of nitrogen – ammonium, can be the better nitrogen source for plant growth. However, the application of ammonium as the sole nitrogen source leads to developmental disorders of most crop plants. We propose that mitochondria may be engaged in regulating the redox homeostasis in plant tissues during ammonium toxicity to improve oxidative metabolism.

The role of additional mitochondrial electron transport chain components in reducing oxidative stress under ammonium nutrition was determined with the use of transgenic *Arabidopsis thaliana* plants suppressing external NAD(P)H dehydrogenase NDB1. The necessity of electrons to enter the mitochondrial electron transport chain through the NADH dehydrogenase was analyzed in complex I defective *frostbite1* mutants. Moreover, a consequence of the catabolism of carbohydrates being directed through glycolysis to face respiration is the accumulation of methylglyoxal, which derivatives need to be detoxified in mitochondria. It may be concluded that plant mitochondria are key organelles in overcoming ammonium toxicity.

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O.17 Role of mitochondrial alternative oxidase in successful anhydrobiosis of tardigrade

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Tardigrades are water dwelling, eight legged, segmented invertebrates which have the ability to withstand almost complete drying (dehydration) by tun formation. This adaptation for periodic drying occurs naturally in their environment and is known as anhydrobiosis. At present, the recovery from the tun stage to the active stage is the only attainable evidence of successful anhydrobiosis. The role of mitochondria in this process is still discussed. It is known, however, that tardigrades in tun stage show tolerance to high concentrations of potassium cyanide that imposes the role of mitochondrial alternative oxidase (AOX). The protein has been shown to be important for cell survival in stress conditions. AOX activity has been reported for plant, fungal and protist mitochondria but the animal AOX functionality is still a matter of debate. Accordingly, analysis of genomes available for tardigrade species including *Milnesium tardigradum* (our model organism) indicate the presence of AOX encoding gene. We also observed that BHAM (AOX inhibitor) delayed recovery from the tun stage to active life and influenced tardigrade respiration. The obtained results suggest important role of AOX in tardigrade mitochondria and contribution of the mitochondria functioning to successful anhydrobiosis.

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Posters

P.1 Cardioprotective flavonoids as a modulators of potassium channels

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Potassium channels such as K_{ATP}, BK_{Ca} or Kv1.3 have been found in the inner mitochondrial membranes of different cells types. It is considered that potassium channels regulate the mitochondrial membrane potential, respiration, matrix volume and calcium homeostasis. There are hypothesis that mitochondrial e.g. BK_{Ca} channels play an important role in ischemic preconditioning. It was also shown that mitochondrial potassium channels are potential targets for some flavonoids in the anti-ischemic strategies.

Our pervious study showed functional properties of the BK_{Ca} channel in mitochondria of endothelial cells (EA.hy 926). Large conductance (270 pS), voltage dependence, a high open-state probability at positive potentials, sensitivity to Ca^{2+}, NS1619 (a BK_{Ca} channel opener) and paxilline (BK_{Ca} channel inhibitor) indicate similarity to the mammalian BK_{Ca} channel. Previously, these channel was also discovered in glioma, brain, skeletal muscle and cardiac.

In the current study, single channel activity of the mitoBK_{Ca} channel was measured after patch-clamp of the mitoplasts isolated from endothelial cell line (EA.hy 926). We show detailed data describing regulation of the mitoBK_{Ca} channel by the cardioprotective flavonoids (luteolin, quercitin and cyanidin). We have observed that opening probability of the channel increased from 0.15 in the control conditions (100 μM Ca^{2+}) to 0.26 after application of cyaniding in micromolar concentration range.

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P.2 Biochemical and phylogenetic studies of mitochondrial post-transcriptional regulators from the FASTK family

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We study the structure and function of the Fas-activated Serine-Threonine Kinase (FASTK) protein family and its relatives. Six paralogs can be found in the human mitochondria where they contribute to the regulation of the cellular respiration at the post-transcriptional level. FASTK proteins contain predicted N-terminal helical regions and a C-terminal putative PD(D/E)XK nuclease domain overlapping with another small domain called RNA-binding domain abundant in Apicomplexans (RAP). The structure of any of the FASTK proteins is not known, and no reliable high-sequence-identity homologues with known structure could be identified. We are using structural biology methods and RNA-binding assays, together with a phylogenetic analysis of proteins with RAP domains, to determine the structure and function of FASTK proteins. We report first successful protocols for obtaining soluble constructs of several human FASTK homologs, which should enable their biochemical characterization. Our bioinformatics analyses detect repeat motifs in the helical regions of FASTK homologs in other organisms, and let us identify interesting phylogenetic relationships.

P.3 Reactive oxygen species formation and ubiquinone reduction level in Acanthamoeba castellanii mitochondria

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Acanthamoeba castellanii is a small non-photosynthesizing amoeba, which is frequently used in studying mitochondrial respiratory chain. Coenzyme Q (Q) is an important mobile component of the mitochondrial electron transport chain that takes part in mitochondrial reactive oxygen species (ROS) production, contributing to oxidative stress and damaging mitochondria and cells. On the other hand, Q displays an antioxidant property that protects the cells from harmful ROS. A plant-type respiratory chain of *A. castellanii* mitochondria contains two QH_2-oxidizing pathways, the classical cytochrome pathway and the alternative ubiquinol oxidase (AOX). The aim of our study was to elucidate the relationship between ROS formation and the reduction level of Q pool under different mitochondrial respiring conditions, i.e., at a diverse engagement of Q-reducing pathway (succinate dehydrogenase, complex II) and QH_2-oxidizing pathways (the cytochrome pathway and AOX) in isolated *A. castellanii* mitochondria. The Q reduction level was increased by inhibition of QH_2-oxidizing pathways (complex III, complex IV, or AOX) or through inhibition of oxidative phosphorylation system (ATP synthase or ATP/ADP antiporter). The Q pool was shifted to a more oxidized state through inhibition of the Q-reducing pathway (substrate dehydrogenase) or by stimulating the activity of QH_2-oxidizing pathways under uncoupling conditions (the cytochrome pathway) or under GMP-activation (AOX). We measured the Q reduction level under given mitochondrial oxygen consumption and membrane potential conditions in relation to H_2O_2 formation. Our results indicate that membranous Q reduction level is proportional to ROS formation within a defined respiratory path-dependent range.

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P.4 Insights into mitochondrial dynamics in primary fibroblasts derived from patients diagnosed with sporadic form of Alzheimer's disease

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Mitochondria are multifunctional, dynamic organelles, which are continuously undergoing fusion and fission, turnover (biogenesis and clearance of mitochondria), as well as movement along the cytoskeleton. Proper mitochondrial dynamics is crucial for the maintenance of vital functions of the cell and accordingly, its dysregulation can lead to pathological conditions within the cell. Mitochondrial disturbances were observed in the most common neurodegenerative disorders. Detailed mechanisms of these impairments are still lacking. In our study, conducted on primary fibroblasts derived from patients with sporadic form of Alzheimer's disease (AD), we showed diminished mitochondrial turnover, changes of the level of proteins involved in mitophagy and decreased level of factors engaged in biogenesis. Moreover mitochondria in AD cells were functionally older and created diverse mitochondrial network (less fragmented, longer branch length, different number of junctions). Additionally the level of fission proteins were reduced. Investigations of mitochondrial dynamics seems to be important for better understanding the pathogenesis of Alzheimer's disease.

P.5 Nucleo-mitochondrial interactions - retrograde regulation in yeast *Candida albicans*

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We study the regulatory response of the nuclear genome to the mitochondrial dysfunction in *C. albicans*, comparing it to the well-known *S. cerevisiae* pathway. *In silico* analysis showed that both the major effector (*CIT2* gene) and regulator (Rtg2p) of this pathway in *S. cerevisiae* are not found in *C. albicans*. Orthologues of the major transcription factors: Rtg1p and Rtg3p, are, however, present.

Deletion of *CaRTG1* or *CaRTG3* in a strain with functional mitochondria (WT) did not affect its respiratory capacity. On the other hand, when one of these two genes was deleted in a strain with dysfunctional mitochondria - Δ*Caaep3*, deficient in the expression of mitochondrial ATP synthase, the respiratory phenotype was suppressed. In *C. albicans*, one of the alternative respiratory pathways is the alternative oxidase – AOX, which is not present in *S. cerevisiae*. AOX is constitutively expressed at a low level in WT, and its main function is reducing ROS stress in mitochondria. Our RNA sequencing results showed that *CaAOX2* expression level was significantly elevated in Δ*Caaep3*, while in a double mutant Δ*Caaep3* Δ*Cartg1* and Δ*Caaep3* Δ*Cartg3* it was decreased even below the WT level. These results suggest a functional relationship between the alternative oxidase and the retrograde pathway in *C. albicans*.

In *S. cerevisiae*, ScRtg3p and ScRtg1p localize to the nucleus in response to various stresses. In *C. albicans*, these two proteins constitutively localized in nucleus, both in strains with functional, and dysfunctional mitochondria. Our results also suggested that the nuclear localization of CaRtg1p could depend on CaRtg3p.

P.6 Changes in mitochondria morphology in EA.hy 926 cell line upon endoplasmic reticulum stress

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Endoplasmic reticulum (ER) stress is responsible for accumulation of unfolded proteins inside lumen of endoplasmic reticulum network. This phenomena leads to process called unfolded protein response (UPR) which activates three major response pathways related to the PERK, eIRE1α and ATF6 proteins. Mild ER stress leads to changes in protein expression which can have protective effect and it can be consider as a form of cell homeostasis. It is also well known that in the cells there are specific contact points between ER and mitochondria - mitochondria associated membranes (MAM`s) and still this relation is not well established. We study phenomena of ER stress in endothelial human cell line EA.hy926. Endothelium lines the interior of blood vessels such as arteries, arterioles, venules, vein and capillaries and this cell line are responsible for plethora of cardiovascular processes such as vasodilatation, coagulation and atherosclerotic plugs formation. The ER stress was evoke by tunicamycin, thapsigargin and DTT, these compounds have a different mechanism of action. The changes in mitochondrial morphology and theirs metabolic activity were study.

To follow the mitochondrial morphological changes we perform imaging of live EA.hy 926 cells by confocal microscopy. Mitochondria were visualized with MitoTracker® Green fluorescence probe and we also imaging line of endothelium cells with stable expression of fluorescent mCherry protein. From confocal images of mitochondria we calculate length of branches and count how many junctions in the mitochondrial network exist. These parameters give us information about level of fragmentation of mitochondria network. We also determined of early and late stage of apoptosis and necrosis after different time treatment of cells by drugs: tunicamycin, thapsigargin and DTT with Annexin V and Necrosis detection reagent kit.

Our results show that endoplasmic reticulum stress induce by tunicamycin, thapsigargin and DTT influence on morphology of mitochondria in EA.hy926 cell line, and longer incubation time leads to apoptosis and necrosis processes.

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P.7 Identification of novel RNA-binding proteins in human mitochondria with focus on poly(A)-binding proteins

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Binding of proteins to RNA is crucial for every step of gene expression and RNA metabolism. For instance, interaction of poly(A) binding proteins with polyadenylated nucleus encoded transcripts influences their stability and translation. It is known that mitochondrial mRNAs also possess stable poly(A) tails, however mitochondrial poly(A) binding proteins are unknown.

The project's aim is to identify novel human mitochondrial RNA binding proteins with particular focus on poly(A) binding proteins. In accomplishing this goal I follow two research strategies. The first, which is a candidate gene approach, is focused on investigating putative mitochondrial RNA binding proteins identified based on available literature. In the second approach mitochondrial protein extracts are subjected to affinity chromatography (poly(A) conjugated beads) and then purified proteins are identified by mass spectrometry. Initial experiments showed relatively high background. After a series of optimization experiments I decreased the background and improved the approach. A group of putative mitochondrial RNA binding proteins has been identified and some of them were selected for further studies.

P.8 A defect in processing of 18S and 5S rRNA precursor in *Arabidopsis* with a decreased level of mitoribosomal protein S10

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Apart from protein synthesis, many of the ribosomal proteins are shown to be involved in other cellular functions. We found that silencing of the nuclear gene encoding mitochondrial ribosomal protein S10 in *Arabidopsis* leads to unequal level of two co-transcribed mitochondrial rRNA: 18S and 5S. Since in bacteria and mammals the ribosomal S10 protein is involved in control of transcriptional and posttranscriptional metabolism of rRNA (Greive et al., 2005; Doherty et al., 2010), we decided to test if the S10 protein in *Arabidopsis* mitochondria is also involved in biogenesis of rRNA.

Using real time and circularized RT-PCR we found that besides an increased level of mature 18S and 5S rRNAs in *rps10* mitochondria two populations of their precursors were also accumulated. These precursors are also present in the wild type, but at a very low level. First population consists of poliadenylated, full-length precursors of 18S and 5S rRNA while the second population is heterogeneous and contains shorter forms. All these shorter forms have 18S rRNA, but not 5S rRNA. Furthermore, 18S rRNA is linked with different length of ITS (intergenic sequence) and only a part of shorter forms is polyadenylated . Based on obtained data we postulate that the S10 protein is required for a proper maturation of pre-18S rRNA in *Arabidopsis* mitochondria.

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P.9 Oxygen consumption and mitochondrial membrane potential in isolated endothelial mitochondria can be modulated by flavonoids

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The cardioprotective properties of naringenin, quercetin, cyanidin and luteolin have been previously shown. These flavonoids show antihypertensive and antiatherosclerotic effects. They prevent platelet aggregation and progression of endothelial dysfunction. Some of these effects can be assigned to the antioxidant properties of flavonoids. However, numerous studies suggest that the key role in flavonoid-induced protection is due to mitochondrial potassium channel activation. In this study, we have examined the influence of flavonoids on isolated endothelial mitochondria. The rate of succinate-sustained non-phosphorylating respiration was enhanced in response to naringenin, naringenin chalcone, quercetin and cyanidin in mitochondria isolated from EA.hy926 cells. What is interesting, the effects of naringenin and cyanidin were partially reversed by iberiotoxin, a mitochondrial large-conductance calcium-activated potassium channel (mitoBK_{Ca}) blocker. Naringenin and cyanidin also induced iberiotoxin-sensitive, modest (~ 1mV) depolarization of mitochondrial membrane potential that was determined with a TPP^+-specific electrode. Thus, our results imply that flavonoids modulate oxygen consumption and membrane potential of endothelial mitochondria via their interaction with mitochondrial BK_{Ca} channel.

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P.10 Mitochondria and anhydrobiotic tardigrade survival

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Water availability is one of the most important factors for terrestrial life. However terrestrial habitats may endure occasional drought that can be overcome by the organism ability to undergo anhydrobiosis. Thus, the term anhydrobiosis, from the Greek for “life without water”, is defined as dehydration/desiccation tolerance. It is also defined as an organized state and as such it requires some form of energy supply that suggests mitochondria contribution. Accordingly, it can be assume that mitochondrial role in maintaining proper course of dehydration/desiccation process includes ATP synthesis based on the oxidative phosphorylation and efficient reactive oxygen species (ROS) scavenging mechanisms. In the case of animals the phenomenon has been reported for some small invertebrates including tardigrades being the most known example. Therefore we decided to estimate the impact of anaerobic conditions on anhydrobiotic tardigrade (so-called “tun”) survival as well as to assess mitochondrial coupling, the levels of mitochondrial ROS and storage cell lipid content using the proper fluorescence dyes. For the purpose we used anhydrobiotic tardigrade *Paramacrobiotus* cf. *richtersi* as a model. The obtained results indicate that the tardigrade tun survival needs mitochondrial coupling and duration of the tun stage affects mitochondrial ROS levels and the amount of lipids in storage cells. The latter is negatively correlated with the tun ability to return to the active state and survive anaerobic conditions. Thus, these results support the important role of mitochondrial activity in successful anhydrobiosis.

The work was supported by the research grant of National Science Centre, Poland, NCN 2016/21/B/NZ4/00131.

P.11 Pet127p and Dss1p exoribonucleases are responsible for intron degradation, changes in steady-state expression levels and mitochondrial RNA maturation in *Candida albicans*

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Pet127p and Dss1p are two exoribonucleases that play diverse roles in fungal mitochondrial RNA metabolism. So far they have only been studied in *S. cerevisiae,* where phenotypic pleiotropy due to the loss of mtDNA stability obfuscates their functional analysis, and in *S. pombe,* where the phenotypes are much less pronounced. Here we studied their function in *C. albicans* by RNA-seq of the mitochondrial transcriptome in wild type, Δ*pet127* and Δ*dss1* mutant strains.

Pet127p in *S. cerevisiae* is considered to play a role in mitochondrial RNA maturation and degradation via its putative 5’-3’ exoribonucleolytic activity, which has never been directly demonstrated. Our results in *C. albicans* show a very significant accumulation of the large ribosomal subunit rRNA introns accumulation in Δ*pet127* mutant. Accumulation of 5’ ends of both introns, however, did not affected the proper splicing and rRNA maturation. Dss1p is a part of MtEXO, the mitochondrial exosome complex responsible for 3’-5’ RNA degradation and mtRNA maturation. RNA-seq of Δ*Dss1* mutant shows accumulation of both rRNA’s, intergenic RNA (pervasive transcription), unprocessed 3’ ends of Primary Transcript Units (TU’s) and significant decrease of amount of mature tRNA which lead to respiratory defect and inability to grow on non-fermentable carbon source.

P.12 Synthesis of ROMK1/2 protein in *Eschericha coli* for functional studies

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ROMK2 (Kir1.1b) is a mitochondrial isoform of plasmalemmal ROMK1 channel (Kir1.1a), which is missing the first 19 amino acids. It is believed that ROMK2 is a part of the mitoK_{ATP} channel. ROMK2 protein forms homotetrameric channels in which each subunit consists of cytoplasmic N- and C-termini and a core region of two transmembranes helices flakning a P-loop segment.

To obtain purified ROMK1/2 channels for further studies, we expressed the ROMK1/2 protein in *Eschericha coli*. The sequence of several expression tags was fused to codon-optimized ORFs or chimera between cytoplasmic N- and C-termini of ROMK1/2 and transmebrane part of bacterial potassium channel KirBac1.3. These tags (MISTIC, SUMO and OMPF) could assist membrane insertion and folding of proteins in bacteria. The fusion proteins contained also affinity tags for purification (N- or C- terminal 6xHis). The ROMK1 with C-terminal 6xHis construct was chosen for further studies because it exhibited the highest membrane expression level and low degradation. We screened several detergents and n-dodecyl β-D-maltoside (DDM) was selected for protein purification.

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P.13 Characteristics of mitochondrial potassium channel formed by the BK-DEC splice variant

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Ischemia of brain or heart tissue is the one of the most common causes of death in most Western countries. In the inner mitochondrial membrane several potassium channels have been identified to have cytoprotective function during ischemic event. One of them is mitochondrial large conductance calcium activated potassium channel (mitoBKCa). It was found that activation of mitoBKCa preserves brain and heart muscle cells. Recently, the molecular identity of the mitochondrial BKCa channel was described. The BK-DEC splice variant of BKCa-type channels α subunit has been demonstrated to localize in mitochondria. However it is not known whether this isoform is able to form a functional pore in mitochondria. In our study we used HEK293T cells transiently transfected with cDNA encoding the BK-DEC splice variant. Electrophysiological recordings with use of mitoplast isolated from transfected cells revealed the presence of the large conductance and voltage dependent ion channel. This type of channel was not present in mitoplasts isolated from untransfected cells. We found that the recorded channel showed all basic pharmacological properties typical for the mitoBKCa channels described previously. The channel was Ca2+ sensitive and inhibited by a well-known mitoBKCa channel inhibitor - paxilline. Additionally, kinetics and conductance of the observed channel were very similar to the mitoBKCa channel’s. Based on that data we conclude that the BK-DEC splice variant forms a functional channel in the inner mitochondrial membrane of HEK293T cells.

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P.14 Mitochondrial-related mutation G171H in cytochrome *b* of *Rhodobacter capsulatus* cytochrome *bc1* suppresses generation of superoxide

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Cytochrome bc_1 plays an essential role in electron transport chain. It takes part in proton motive force formation. Because only cytochrome *b* is encoded by mitochondrial DNA, this subunit is more prone to mutations compared with other subunits encoded by nuclear DNA. Some of these mutations are related to mitochondrial disorders in humans because of the reduced efficiency of energy conversion. In this work we examined a mutation Y155H, which was found in cytochrome *b* in a Prader Willi Syndrome patient. Using a purple bacterial model we studied an analogous mutation G171H in *Rhodobacter capsulatus*. The enzyme with this mutation shows reduced activity in the wide range of pH, however the K_M, defining the affinity of the enzyme to quinone, is the same for G171H and wild type protein. Interestingly, we found that the superoxide production is dependent on the duration of enzymatic reaction. So far, this phenomenon has not been described in any other mutants of cytochrome bc_1. For the first few seconds the mutant displays reduced free radicals production in comparison to the wild type. As free radicals are engaged in cellular signaling, we propose that the molecular effect of Y155H mutation is a delayed response to redox state of the membrane.

P.15 CaRMD9 – a PPR protein of petite negative yeast *Candida albicans* required for stability of multiple mitochondrial transcripts

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Candida albicans is a facultatively anaerobic and potentially pathogenic yeast, causing a variety of infections, especially in patients with compromised immune system. *C. albicans* mutants with respiratory deficiency are viable, but they do not tolerate mitochondrial DNA loss (petite negative), providing the opportunity to investigate primary effects of deletions of genes essential for mitochondrial genome expression. The mitochondrial genome of *C. albicans* encodes NADH dehydrogenase subunits (complex I) absent in mtDNA of *Saccharomyces cerevisiae*, but present in human mtDNA. For this reasons we use *Candida albicans* as a fungal model of mitochondrial gene expression, particularly to investigate nuclear regulators of mitochondrial RNA turnover.

PPR proteins are known as organellar factors involved in transcription, RNA processing and stabilization, transcript end maturation, splicing, and translation. CaRmd9p belongs to PPR family and is the only *C. albicans* ortholog of paralogous ScRmd9p and ScRmd9Lp found in *S. cerevisiae*.

Deletion of *CaRMD9* results in respiratory deficiency. Cells with reconstituted *CaRMD9* gene are however respiratory proficient suggesting that phenotype of *ΔCarmd9* is completely reversible. Molecular phenotype of *ΔCarmd9* is similar to *Δrmd9* in *S. cerevisae*. We observe changes in the small ribosomal subunit RNA (RNS), and a decreased level of all mitochondrial transcripts, except COX2 and large ribosomal subunit RNA (RNL). Interestingly, these transcripts originate from the same policistronic transcription unit. Reconstitution of native *CaRMD9* restores the level of all mitochondrial transcripts .

P.16 Time-course of changes in the mitochondrial ETC protein content, muscle oxidative stress markers and antioxidant capacity in the soleus muscle of rats during endurance training

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Background: Prolonged endurance training enhances mitochondrial biogenesis in the trained skeletal muscles, but the underlying mechanism is not fully understood. Recent studies suggest a role of reactive oxygen species in the training-induced intensification of mitochondrial biogenesis. The aim of this study was to investigate the relationship between training-induced enhancement of mitochondrial biogenesis and the markers of muscle oxidative stress studied at 1, 2, 4 and 8 weeks of endurance training (ET). **Methods:** Mitochondrial biogenesis was assessed based on the expression of the electron transport chain (ETC) proteins (C-III, C-IV, C-V ETC complexes) using Western immunoblotting (WB). Glutathione level (muscle reduced glutathione $[GSH]_m$ and oxidized $[GSSG]_m$) and superoxide dismutase 2 (SOD2) expression were detected using LC/MS/MS and WB, respectively. **Results:** We have found an attenuation of total glutathione level $[GSH/\ 2xGSSG]_m$ as soon as after 1 week of ET, which remained decreased until the 8^{th} week of ET. However, an increase in SOD2 expression, was visible not earlier than at the 8^{th} week of ET. Moreover, an increase in ETC proteins content including key-one cytochrome-c oxidase (C-IV complex) has been found not sooner than at the 8^{th} week of ET. **Conclusions:** The dissociation between the immediate increase in oxidative stress markers and the delayed enhancement of the expression of ETC proteins during the time-course of training questions the postulated key role of oxidative stress in training-induced increase in mitochondrial biogenesis, as judged based on the ETC protein content.

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P.17 An influence of the lack of mitochondrial proteases AtFTSH4 and AtOMA1 on growth and development of *Arabidopsis thaliana*

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Mitochondria as organelles of endosymbiotic origin have their own well-developed proteolytic system. Here, we focus on two metalloproteases of the mitochondrial inner membrane in *Arabidopsis thaliana*, AtFTSH4 and AtOMA1. AtFTSH4 is an ATP-dependent protease in contrast to AtOMA1, which does not require ATP for its proper functioning. AtFTSH4 belongs to the *i*-AAA protease family, of which the catalytic site is oriented toward the intermembrane space. Topology of AtOMA1 is not confirmed yet, however, it is known that catalytic site of OMA1 homologues in mammals and yeast is also in the intermembrane space. We show that the lack of one of the above-mentioned proteases does not lead to the significantly altered phenotype of *A. thaliana* grown on artificial medium under long-day (LD) photoperiod and 22ºC. On the other hand, *ftsh4* and *oma1* plants that are exposed to continuously higher temperature (30ºC) reveal impaired root and rosette growth. Surprisingly, the lack of both AtFTSH4 and AtOMA1 causes dramatically altered phenotype under optimal conditions (LD, 22ºC). Phenotype analysis showed that double *ftsh4oma1* mutant germinates significantly slower than wild-type (WT) or single mutants (*ftsh4-1* and *oma1-1*) and its growth is arrested at the early-seedling stage (cotyledons stage). It indicates that presence of at least one of these proteases is required for normal growth of *A. thaliana* under optimal conditions and suggests that AtOMA1 and AtFTSH4 may have overlapping functions and control the same substrates.

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P.18 Studies of *PRKN, PINK1, TOMM20* and *TOMM70* in patients diagnosed with Parkinson's disease

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Parkinson's disease (PD) is associated (5 - 10%) with mutations in many genes, like PRKN and *PINK1* found to participate in the quality control of mitochondria. *PRKN* encodes Parkin protein, that is E3 Ub ligase and product of *PINK1* is a serine/threonine protein kinase. Loss of transmembrane potential in damaged mitochondria leads to the accumulation and activation of PINK1 on the mitochondrial outer membrane. The activated PINK1 recruits Parkin and stimulates Parkin E3 activity, promoting mitophagy. Mutations in these genes cause early-onset PD. It is assumed that the PD might also be caused by the dysfunction of the protein import machinery whereas TOM complex seems to be the most important for recognition (by its two receptors; Tom20 and Tom70 and translocation of the most of incoming precursor proteins. In this study we partially investigated genes *PRKN, PINK1, TOMM20* and *TOMM70* in the 30 PD patients. In the case of *PRKN* we obtained point mutation in exon 4 at the site of c. 454 G> T, which causes the change of the amino acid lysine (K) to methionine (M), in the RING0 domain. This mutation was observed at 3 samples, as a heterozygous genotype. For the *PINK1* was obtained one SNP g.2022C<T in the 4 heterozygous patients. We find no mutation for exons 3 and 4 of *TOMM20*. For gene encoding *TOMM70* we found some polymorphisms in the 3'UTR region: *rs1801865* polymorphism in the 16 heterozygous patients, *rs1045610* polymorphism in the 6 heterozygous patients and in the 3 homozygous patients.

In the light of the obtained results, further studies have to be followed as for other fragments of the studied genes as well as for more patients. The continuation of these studies is crucial for better understanding of the mitochondria function in PD biogenesis.

P.19 The mitochondrial cytochrome *bc*$_1$ complex in the determination of ROS production

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The mitochondrial cytochrome *bc*$_1$ complex, also known as complex III or ubiquinol cytochrome c oxidoreductase is a key component of the electron transport chain in eukaryotic mitochondria and several prokaryotic organisms. It has been regarded as one of the main superoxide anion generation sites, with its implicated involvement in apoptosis, aging process and cellular signalling. Superoxide radical production at the catalytic site of ubiquinol oxidation in cytochrome *bc*$_1$ is commonly linked with formation of the unstable ubisemiquinone. Here we show the significant differences in the rate of ROS production and catalytic properties of mitochondrial cytochrome *bc*$_1$ in comparison with its prokaryotic equivalent isolated from *Rb. capsulatus.* We also show the effectiveness of different ubiquinone analogues and their influence on complex III activity and radical generation. By mimicking heme *c*$_1$ redox potential of mitochondrial cytochrome *bc*$_1$ via the introduction of specific point mutation, we show how the mitochondrial phenotype can be recreated in our prokaryotic model and how it influences ROS generation.

P.20 Binding of an AU-rich motif in the SSU mitochondrial rRNA by the yeast PPR protein Dmr1/Ccm1 – mechanistic and evolutionary insights.

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PPR proteins are large family of RNA binding factors present in all Eukaryotic lineages. They perform a variety of roles at the posttranscriptional stage of organellar gene expression [1]. PPR proteins are also significant determinants of evolutionary nucleo-organellar compatibility. Some plant PPR proteins recognize their RNA substrates via a straightforward modular code [2]. To date, no such code was recognized in non-plant organisms. Dmr1p/Ccm1p is a *S. cerevisiae* PPR protein involved in the stabilization of 15S ribosomal RNA (and is thus essential for mitochondrial DNA expression and mtDNA stability) [3].We found that in vitro Dmr1p specifically binds a motif composed of multiple AUA repeats. Such motifs occur twice in the 15S rRNA sequence as the minimal 14 nucleotide (AUA)4AU or longer (AUA)7 variant. Short RNA fragments that contain such motif are protected by Dmr1p from exoribonucleolytic activity *in vitro*.

Presence of the identified motif in mtDNA of different yeast species correlates with the compatibility between Dmr1p orthologues from these species and *S. cerevisiae* mtDNA. RNA recognition by Dmr1p is likely based on a rudimentary form of a PPR code specifying U at every third position, and depends on other factors, like RNA structure. It is also likely that the PPR code in *Opisthokonta* is limited to distinguishing between purines and pyrimidines.

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P.21 Metabolic markers of active and anhydrobiotic tardigrades

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Tardigrades are small invertebrates that inhabit terrestrial, freshwater and marine environments throughout the world and are considered most resistant animals on our planet. Together with nematodes and rotifers, tardigrades are best known animals capable of anhydrobiosis, which is known as a desiccation tolerance that denotes the ability to survive almost complete dehydration without sustaining damages. The analysis of available data suggests that anhydrobiosis success requires proper carbohydrate and lipid metabolism. Therefore we decided to compare metabolic profiles of active and anhydrobiotic tardigrades (so called “tuns”) differing in anhydrobiosis capability. In our study we used active and anhydrobiotic specimens of terrestrial tardigrade - *Echiniscus testudo* (Doyère, 1840) and active specimens of limno-terrestrial *Hypsibius exemplaris* Gąsiorek, Stec, Morek & Michalczyk, 2018. To follow putative alterations of metabolism we applied a metabolomic approach, i.e. untargeted metabolomic profiling based on gas chromatography-mass spectrometry (GC-MS). The settled methodology leads to detection of different metabolites and allows for determination of metabolic differences between the active and tun stages. The detected unscrambled metabolites represented mainly amino acids, monosaccharides, carboxylic acids, membrane lipids and some products of the tricarboxylic acid (TCA) cycle. The main tool is to provide metabolic markers allowing discrimination between active and anhydrobiotic tardigrades.

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P.22 A novel potassium channels in the human bronchial epithelial mitochondria

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Mitochondria have been recognized for their multifunctional roles in energy transduction, ion transport, signaling and cell death. It has been observed that potassium flux through the inner mitochondrial membrane regulates synthesis of the reactive oxygen species, affects the mitochondrial volume and changes both the mitochondrial membrane potential and the transport of calcium ions into the mitochondria. Additionally, it has been shown that activation of mitochondrial potassium channels (e.g. mitoBK$_{Ca}$) protects against cell death during myocardial infarction or cerebral hypoxia.

Our studies using the patch-clamp technique proves the presence of two different potassium channels in the inner mitochondrial membrane of human bronchial epithelial cell line (16HBE14σ-). We identified the activity of rectifying potassium channel and large-conductance Ca^{2+}-regulated potassium channel (mitoBK$_{Ca}$ channel). Using reverse transcriptase-PCR, we detect mRNA transcript for KCNJI (ROMK) channel as a molecular component of the mitoK$_{ATP}$ channel. Moreover, the protein of ROMK was also observed by Western Blot analysis. Additionally, it has been confirmed the presence of α-subunit and modulatory β-subunits of BK channel proteins (Western Blot analysis) and genes expression (reverse transcriptase-PCR analysis).

We believe that our findings of the potassium channels of epithelial mitochondria, it will help us better understand of their role in global protective mechanisms.

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P.23 Targeting mitochondrial DNA repair for novel anti-cancer therapies

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Although base excision repair (BER) pathway can be readily detected in mitochondria, the molecular mechanism by which mitochondrial DNA (mtDNA) is repaired is not well understood. Recently, human mitochondrial EXOG, a membrane bound 5'-exo/endonuclease, has been shown to be crucial for mtDNA repair. Depletion of EXOG causes accumulation of DNA damage in the mitochondria, but not in the nucleus, increases oxidative stress, causes mitochondrial dysfunction, and leads to apoptosis. It has been recently shown that, in contrast to the nuclear DNA, where DNA instability is one of the hallmarks of cancer, intact mtDNA is critical for tumorigenic potential. Because preservation of mtDNA integrity in cancer cells is a key for cancer progression, given its critical function in mtBER, EXOG emerges as a potential chemosensitizing target. The goals of this project are 1) to reconstruct full length EXOG using model membranes to investigate the structure and function of EXOG in its near native conditions and 2) to develop small molecules to modulate and inhibit the activity of EXOG *in vitro* and *in vivo*. Proposed studies will reveal the fundamental biological principles that govern mtDNA repair and provide the opportunity for structure-function guided design of EXOG-specific inhibitors that may act as anti-cancer agents and increase sensitivity to traditional chemotherapeutics.

P.24 Profiling translation on the surface of mitochondria in zebrafish

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Most mitochondrial proteins originate from the cytosol and require active import into the organelle. Almost all mitochondrial proteins can be imported into mitochondria in a post-translational manner. Therefore, for decades the post-translational import was considered the main route of transporting proteins into mitochondria. At the same time, the evidence for alternative routes has been long debated. Recent high-throughput transcriptome and structural studies in yeast have revealed that many nuclear encoded mRNAs and polysomes are associated with the mitochondrial outer membrane, supporting the view of co-translational import. The early stages of mitochondrial protein targeting are still poorly understood. To uncover which nuclear encoded mRNAs are translated on the surface of mitochondria, we performed a high-throughput study to profile mRNAs associated with the mitochondrial surface in zebrafish (*Danio rerio*). The outcome of this study suggests that the co-translational import of mitochondrial proteins in vertebrates is much more widespread than previously expected.

P.25 H_2S effects on the activity of mitoBK$_{Ca}$ channels - dependence on protoporphyrin central metal ion

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Mitochondrial large-conductance calcium-activated potassium channel (mitoBK$_{Ca}$) is one of the main potassium channels localized in the inner membrane of mitochondria. MitoBK$_{Ca}$ channel is formed by a DEC splice variant of KCNMA1 gene and is a tetrameric protein composed of four α subunits. Each α subunit consists of a short N-terminus, seven transmembrane segments and C-terminus containing two RCK (regulating conductance of K^+) domains located in the mitochondrial matrix. Various modulators of the activity of BK$_{Ca}$ channels are known, including activator carbon monoxide and inhibitor heme (Fe(II)-protoporphyrin IX) or its oxidized form hemin (Fe(III)-protoporphyrin IX). It is known that the activity of mitoBK$_{Ca}$, similarly to that of plasmalemmal BK$_{Ca}$ channels is inhibited by hemin, which binds to –CXXCH– motif located between two RCK domains. Hypothetically, H_2S can act as a cytoprotective factor by opening of mitochondrial potassium channels. To test this hypothesis, we performed patch-clamp experiments on mitoplasts derived from mitochondria of astrocytoma U-87 MG cells to measure activity of single mitoBK$_{Ca}$ channels. We applied sodium hydrosulfide (NaHS) as a H_2S donor in combination with various ion-substituted protoporphyrins IX. We observed that NaHS applied alone doesn't change the activity of mitoBK$_{Ca}$ channels. However, it reversibly activates the hemin-inhibited mitoBK$_{Ca}$ channels and irreversibly activates the Sn(IV)-protoporphyrin IX -inhibited channels. These observations are consistent with the reactivity of H_2S towards central metal ion in these protoporphyrins.

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P.26 Missense mutation of FBP2 gene causes reversible early childhood leukodystrophy

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A novel type of leukodystrophy causing severe symptoms in early childhood was identified. Contrary to most other leukodystrophies, patients recovered well in later life. The disease was linked with a heterozygous missense p.V115M mutation in the FBP2 gene. Analysis of recombinant mutated FBP2 protein revealed lower enzymatic activity and thermodynamic stability compared to the wild type. Investigation of fibroblast cultures from patients and healthy kin show abnormal cellular localization and increased ubiquitination of the FBP2 protein. FBP2 is known to protect mitochondria against Ca^{2+} and ROS stress. In fibroblasts carrying p.V115M mutation, we observed abnormal mitochondrial polarization and increased ROS levels. This suggests that the p.V115M mutation prevents proper folding of FBP2 leading to its degradation and/or general unfolded protein response as well as impaired mitochondrial function. Certainly, FBP2 plays a crucial role in the development of brain white matter. However, the precise mechanism linking p.V115M mutation effects to the demyelination observed in patients remains to be discovered. The cause of reversal of the symptoms remains unknown as well. Researching this topic further can help bring new treatments not only for this particular type of leukoencelopathy but for other similar diseases as well, in addition to giving new insight on post-natal development of the human brain.