Session 5: Molecular and cellular bioenergetics

Lectures

L.05.1

Protein homeostasis and functioning of mitochondria under optimal and stress conditions in plants: the relevance of mitochondrial proteases

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Organisms have developed specific mechanisms known as the mitochondrial protein quality control system, essential for maintaining a healthy mitochondrial population. Inner membrane metallopeptidases *i*-AAA and OMA1 are of particular importance. In Arabidopsis plants, *i*-AAA protease FTSH4 and OMA1 exhibit conserved and plant-specific functions. Whereas the absence of either protease does not affect plant growth under optimal conditions, it leads to morphological alterations under moderate heat stress. The knowledge of protein substrates is vital in understanding protease function. In our work, to identify potential substrates and interaction partners of FTSH4 and OMA1 and to characterize mitochondrial proteomes of *ftsh4* and *oma1* mutants, various proteomic approaches such as co-immunoprecipitation, *i*TRAQ, COFRADIC, and the analysis of insoluble protein aggregates induced by heat have been applied. Our data show that both proteases prevent mitochondrial protein aggregation and that the mitochondrial proteomes of the *ftsh4* and *oma1* plants are similarly adapted to moderate heat stress. Furthermore, under optimal and moderately elevated temperatures, FTSH4 and OMA1 play roles in the quality control of protein carriers and are essential for maintaining mitochondrial morphology and inner membrane ultrastructure.

Acknowledgements

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L.05.2

Potassium ions traffic in mitochondria

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Mitochondrial potassium (mitoK) channels play an important role in mitochondrial physiology. Activation of mitoK channels protects brain and cardiac tissue against injury induced by ischemia reperfusion. Mitochondrial, large conductance calcium activated potassium (mitoBK$_{Ca}$) channel was found in various tissues including brain, cardiac tissue and cancer cells. The basic properties of this channel are similar to other BK$_{Ca}$ channels, including those from the plasma membrane or endoplasmic reticulum. Activation of the mitoBK$_{Ca}$ channel leads to influx of K^+ into the mitochondrial matrix which induces depolarization of the inner mitochondrial membrane and modulates oxygen consumption, and synthesis of reactive oxygen species. Previously, we suggested a functional and perhaps structural interaction between respiratory chain complexes and the mitoBK$_{Ca}$ channel in glioma cells. Our recent studies have shown that loss of the channel leads to deregulation of redox homeostasis in mitochondria. In addition, our ongoing research revealed novel intracellular BK$_{Ca}$-type channel interactions, which may be of importance for a better understanding of the role of these channels in cell physiology and mechanisms of cytoprotection.

Acknowledgements

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Oral presentations

O.05.1

Stress adaptation in mitochondria affected by plastic particles

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Plastics play fundamental role in modern living, it is a useful material ubiquitously present in our everyday lives. Plastic waste degrades and gets reduced to micro- or nanoparticles (MP or NP). It is clear that the air pollution with NP is a global problem. Inhaling MPs and NPs can cause asthma or allergies. Moreover, inhaled NP possess the ability to enter the cell. This study is innovative because so far the influence of inhaled plastic on human health was not broadly researched. Mitochondria are often referred to as the powerhouses of the cell. Yet, their function go beyond energy generation. Mitochondria play roles in cell signaling, calcium homeostasis, apoptosis and other functions. In this project we investigate how the NP pollution affects mitochondrial stress, how does it activate and modulate pathways of mitochondrial retrograde signaling and how mitochondria adapt to stress. In our research model we are using bronchial epithelial cells, healthy (BEAS-2B) and cancerous (A459). The effect of plastic NPs is evaluated after short-term treatment. Here we present the effect of a single dose of NP on mitochondrial morphology and ROS levels. In the future stress effects on the inner mitochondrial membrane potential, mitochondrial age or calcium uptake will be investigated in order to gain a full picture of mitochondrial changes in response to the plastic NPs treatment.

O.05.2

Characterization of the mitochondrial large-conductance calcium-activated potassium channels in rodent cardiomyocytes

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Oxidative metabolism in mitochondria is regulated by the ionic homeostasis. Mitochondrial membranes are equipped with several different ion channels and transporters that are highly efficient and dynamic, what enables regulation and maintaining of ionic homeostasis. Every disruption in this homeostasis causes mitochondrial dysfunction and eventually contractile failure. Moreover, mitochondrial dysfunction during acute ischaemia/reperfusion injury is a critical determinant of cell death following acute myocardial infarction, and therefore, mitochondrial ion channels are known to influence cell death and survival. Activity of large-conductance calcium-activated potassium channels (mitoBK$_{Ca}$ channels) was reported to correlate with increased cytoprotection of cardiomyocytes. Presence and role of mitoBK$_{Ca}$ channels were described already about 20 years ago with guinea pigs (GP) ventricular cells. Interestingly, these channels in GP cardiac cells were reported to have twice lower molecular mass than classical BK$_{Ca}$ channels (55 *vs.* 110 kDa). Herein, presence and activity of mitoBK$_{Ca}$ channels in GP cardiomyocytes were confirmed. Interestingly, our analysis with APC-021 and APC-107 antibodies suggest additional isoforms of mitoBK$_{Ca}$ channels of intermediate mass. Moreover, molecular characterization of α and β subunits was deepened by RT-qPCR analysis and experiments with mice and rat cardiomyocytes.

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O.05.3

The statins atorvastatin and simvastatin induce adaptations of aerobic metabolism in astrocyte cells

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Statins are cholesterol-lowering drugs that block the mevalonate pathway, which is responsible for coenzyme Q (Q) synthesis. The aim of our study was to investigate the effects of 6-day exposure to atorvastatin and simvastatin on oxidative metabolism of cultured rat astrocyte cells (CTX TNA2). Both statins (200 nM) reduced production of reactive oxygen species and did not change level of oxidative stress markers (lipid and protein peroxidation markers). In addition, statins induced increased oxidative mitochondrial capacity and mitochondrial biogenesis. Moreover, by blocking the mevalonate pathway, statins caused a significant decrease in the level of Q, thereby depriving astrocyte cells of an important antioxidant and mitochondrial electron carrier. This resulted in impairment of mitochondrial respiratory function in astrocyte cells. A decrease in mitochondrial respiration occurred with carbohydrate-catabolism reducing fuels (glucose and pyruvate) in statin-treated intact endothelial cells. The oxidation of the strongest substrate (glutamine) was not changed. The observed changes in oxidative metabolism caused a decrease in ATP levels in statin-treated cells. Thus, statins modulate the energy metabolism of astrocyte cells, leading to alterations in the cellular energy state, coenzyme Q redox balance, and mitochondrial respiratory function.

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O.05.4

On the function and mechanism of alternative complex III replacing cytochrome *bc* in bacterial respiratory chain

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Alternative complex III (ACIII) is a membranous complex that catalyses quinol oxidation, the function of enzymes from cytochrome *bc* family. Interestingly, it is evolutionarily unrelated and structurally different from them. The molecular structure of ACIII, resolved by cryo-EM, revealed a supercomplex with aa_3 oxidase and highly intriguing architecture of cofactors suggesting different routes for electrons.

To get insights into the mechanism of action of ACIII we constructed genetic system that allowed for deletion studies of ACIII subunits. We successfully deleted ActA and ActE subunits and heme-containing mobile domain of ActA (mdA) as the ones involved in the catalytic mechanism. The results allowed us to propose new model of electron transfer within this enzyme. Our observations indicate that ActE is not required for electron transfer between ACIII and cytochrome aa_3 and mdA heme is the sole donor of electrons to aa_3 oxidase. Moreover, we examined properties of the redox centres of the ACIII by EPR spectroscopy and redox titrations.

The results of this work help us to define the electron transfer paths connecting ACIII with aa_3, and provide first insight into the functional organisation of the supercomplex.

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O.05.5

The role of human VDAC paralogs under oxidative stress induced by the absence of superoxide dismutases

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Superoxide dismutases (SODs) are enzymes that protect cellular functions by scavenging harmful superoxide radicals. They are located in cytoplasm (SOD1) including mitochondria (SOD1 and mainly SOD2), and contribute to redox balance by maintaining the structural integrity of mitochondrial components. Dysregulation of SODs leads to mitochondrial dysfunction and contributes to related diseases. The human voltage-dependent anion channel (VDAC) protein, located in the mitochondrial outer membrane, serves as a key regulator of metabolic processes by performing the transport of metabolites and inorganic ions across the mitochondrial outer membrane. Additionally, VDAC interacts with various redox-active molecules, including reactive oxygen species (ROS) and antioxidant proteins, influencing cellular redox status. It is proposed that by affecting the flux of ROS, VDAC helps to diminish oxidative stress and enhance cellular homeostasis. We have established a yeast model to study the interplay between each of the three human VDAC paralogs (VDAC1 - VDAC3) and SOD1 and/or SOD2. We analyzed parameters such as cell viability, superoxide anion radical levels as well as mitochondrial structure and functionality by application of growth tests, quantification of ROS production and mitochondria imaging by confocal fluorescence microscopy. The obtained results shed new light on understanding the role of individual VDAC paralogs in the cellular response to oxidative stress.

Poster

P.05.2

Mitochondrial BK_{Ca} channels in senescent vascular smooth muscle cells

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Large-conductance calcium-activated potassium channels (BK_{Ca} channels) play an important role in a negative feed-back loop on depolarization-induced calcium influx and smooth muscle cells (SMCs) contraction. Herein, we confirm presence of its counterparts (mitoBK_{Ca} channels) in the inner mitochondrial membrane. MitoBK_{Ca} channels were shown to protect cardiomyocytes from the ischemia/reperfusion injury. Cardiac events are far more often in aged people, therefore question if those channels are present and active in senescent cells of cardiovascular system seems to be interesting and important. Using few cell lines of human aorta-derived SMCs we induced stress-induced senescence. Then we described changes in mitochondrial network and expression level of the selected genes encoding proteins connected to mitochondrial function between senescent and control SMCs. During the study we detect no changes in the level of mRNA encoding α subunit of BK_{Ca} channels, despite twice lower level of this protein and no activity of the mitoBK_{Ca} channels observed in senescent SMCs. Molecular mechanism of observed changes needs further investigations.

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P.05.3

ROS generation patterns within bc_1 network of heme cofactors

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Cytochrome bc_1 (mitochondrial complex III, CIII) is a key enzymatic complex of the electron transport chain (ETC) linking electron transfer with membranous proton transport in most bacteria and mitochondria. It catalyses the reduction of cytochrome *c* by ubiquinol via sub reaction sequence operating according to the P. Mitchell's Q-cycle. This reaction engages three active sites: quinol oxidation site (the Q_o site), quinone reduction site (the Q_i site) and cytochrome *c* reduction site (C_c site). It involves a series of electron transfer reactions occurring within two distinct cofactor chains: the high-potential c-chain (Rieske cluster and heme c_1) and the low-potential b-chain (heme b_L and heme b_H) connecting Q_o – C_c and Q_i – C_c sites, respectively. This reaction when uncompleted can lead to propagation of side reactions within the system and subsequent generation of reactive oxygen species (ROS). In the present study, we explored electron transfer sequences that were postulated to drive ROS production benefiting from ROS detection method based on a reconstituted hybrid system of bacterial or mitochondrial cytochrome bc_1 coupled to mitochondrial cytochrome *c* oxidase (complex IV, CIV). We analysed *R. capsulatus* mutant strains of hemes *b* (with changed redox potential or a knockout) and heme c_1 (mimicking redox potential of its mitochondrial equivalent) and examined how changes in electron distribution along the cofactor chains impact on ROS production under various conditions.

P.05.4

Loss of BK_{Ca} channel leads to change of mitochondrial redox homeostasis in glioma cells

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Potassium channels present in the mitochondrial membranes play an important role in cellular physiology. Activation of these channels have a cytoprotective effect on tissues exposed to ischemia/reperfusion. In cancer cells, inhibition of these channels leads to an increase of reactive oxygen species and cell death. In U-87 MG glioblastoma, the activity of the mitochondrial large conductance calcium-activated potassium (mitoBK_{Ca}) channel is regulated by the activity of the respiratory chain. Previously, it was suggested that in heart the pore-forming subunit of the mitoBK_{Ca} channel is the isoform of BK_{Ca} channel the plasma membrane and is encoded by the same *KCNMA1* gene.
In our project we used the CRISPR/Cas9 technique to disrupt *KCNMA1* gene in U-87 glioblastoma cells. As a result we obtained cell lines lacking α-subunit of BK_{Ca} channel. Knockout was confirmed by western blot and gene sequencing. Patch-clamp experiments showed loss of the BK_{Ca} and mitoBK_{Ca} channel activity. Furthermore, the lack of a mitoBK_{Ca} channel resulted in an increase in the level of reactive oxygen species in mitochondria. However, the level of mitochondrial respiration did not change significantly.
In conclusion, we show that the pore-forming α-subunit of the mitoBK_{Ca} channel is encoded by the *KCNMA1* gene in U-87 MG cells and the presence of this channel is important for regulating redox homeostasis in the mitochondria.

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P.05.5

Pathophysiological effects of the main protease (Mpro) of SARS-CoV-2 on mitochondria

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Mitochondria are overly sensitive to the stimulus of viral infection. It is currently known that SARS-CoV-2 affects mitochondrial function and consequently modulates the cellular response to infection. Mitochondrial antiviral signaling (MAVS) is affected by viral proteins such as M protein, Orf6 or Orf9b, which lead to inhibition of the innate cellular response. On the other hand, little is known about the effect on mitochondria of another viral protein, main protease (Mpro), which is known to be localized to the outer mitochondrial membrane of the host cell. To investigate this, we constructed a yeast model that, through inducible expression of Mpro in both active and inactive variants, allowed us to perform experiments focusing on cellular bioenergetics. We observed that Mpro disrupts mitochondrial activity and causes an increase in mitochondrial reactive oxygen species. The model we have created may contribute to a more detailed exploration of the role of mitochondria in virus-host interactions, and thus the development of noncanonical therapeutic strategies.

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P.05.6

RNA-seq reveals massive down-regulation of genes in cytoplasmic male-sterile beet plants

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The analysis was performed for 30 table beet plants (*Beta vulgaris* subsp. *vulgaris*, cultivar group *conditiva*) – 15 cytoplasmic male-sterile and 15 with restored male fertility. All pants carried the sterilizing (S-) cytoplasm, in the male-fertile plants its sterilizing effect was suppressed by the presence of a nuclear restorer gene. On average for a single plant 33.5 M paired Illumina reads were obtained (PE100 sequencing). The mean fraction of reads which mapped to the reference genome reached 91.2%. Differential expression between male-sterile and male-fertile plants was found for 775 nuclear genes. Among the genes of at least 4-fold difference in expression over 75% showed higher expression in the male-fertile plants. Analysis of gene ontology revealed that for the cellular component category the best represented differentiating nuclear genes encoded integral membrane proteins. Statistically significant differences in expression were also found for 76 mitochondrial and 11 plastid sequences. The group of differentiating mitochondrial sequences was dominated by those showing increased expression in male-fertile (restored) plants (63 sequences). Examples include genes *rps3*, *atp6* and some exons of *nad* genes.

P.05.7

Nitrogen-containing bisphosphonates disturb calcium homeostasis in isolated endothelial mitochondria

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Osteoporosis is the most common bone-metabolic disorder in postmenopausal women. One of the first-line drugs are bisphosphonates which mechanism of action is to block the mevalonate pathway and inhibit bone resorption. One of the products of this pathway is coenzyme Q, an important antioxidant and mitochondrial respiratory chain electron carrier. During intravenous treatment with bisphosphonates, endothelial cells are reached first by the drug. We studied a direct effect of two nitrogen-containing bisphosphonates, alendronate and zoledronate on respiratory function and membrane potential of mitochondria isolated from EA.hy926 cells. In vitro in lower concentrations both of used drugs did not adversely affect mitochondrial function (phosphorylating and nonphosphorylating respiration, ADP/O ratio, respiratory control ratio). However, after longer (15 minutes) incubation with zoledronate, the mitochondria showed an increase in nonphosphorylating respiration accompanied by a decrease in membrane potential, indicating mitochondrial uncoupling. The addition of zoledronate or alendronate to isolated endothelial mitochondria resulted in disturbances in calcium ion uptake and release, indicating inhibition of the calcium uniporter. Our results demonstrate negative effect on calcium homeostasis in isolated endothelial mitochondria.

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