observed fragmentation of mitochondrial reticulum, oxidative phosphorylation disruption, permeability transition induction, that can lead to kidney cell loss. We also explored the defensive mechanisms such as GSK-3 inhibition, hypoxic preconditioning, mitochondria-targeted antioxidant use which prevented mitochondria damaging and increased cell survival. Partially these effects involve action of nonpathologic quantities of ROS and NO. According to our data during ARF mitochondria are the source, sensors and targets for ROS and NO. Delicate regulation of ROS- and NO-production and utilization is of critical importance to determine the cell fate, which can lead to kidney malfunctioning.

Supported by RFBR grant 08-04-01667.

doi:10.1016/j.bbabio.2008.05.231

S10.6 The NADPH binding on phagocyte NADPH oxidase

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Neutrophils play an essential role in the body's innate defence against pathogens and are one of the primary mediators of the inflammatory response. Neutrophils use a wide range of microbicidal products, such as oxidants, microbicidal peptides and lytic enzymes to kill invading pathogens. The generation of microbicidal oxidants results from the activation of a multiprotein enzyme complex known as the NADPH oxidase, which catalyses the formation of superoxide anion $O_2^{\cdot-}$. The complex is composed of 4 cytosolic subunits (p40phox, p47phox, p67phox, Rac) and two membrane proteins (gp91phox, p22phox). The aim of this work is to clarify the binding of the primary electron donor NADPH on this complex. Two hypotheses have been proposed, either a binding of the nucleotide on a cytosolic component called p67phox or on the membrane flavocytochrome b558. The availability as a recombinant protein of p67phox has allowed a thorough study on this latest. Several methods have been used such as tryptophan quenching, inhibitor analogs and centrifugal sedimentation. None of these techniques has revealed binding of NADPH on p67phox, in opposite to the gp91phox. In conclusion, as expected, the nucleotide binds on the flavocytochrome b558 close to FAD for an efficient electron transfer, the role of p67phox would be to change the affinity of NADPH to regulate the activity of the complex NADPH oxidase.

doi:10.1016/j.bbabio.2008.05.232

S10.7 The phagocytic NAD(P)H-oxidase: Heterologous expression of membrane flavohemoprotein

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The nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase is an essential multiprotein complex present in phagocytic cells of the innate immune system having for function, in response to a bacterial infection, to generate superoxide from a single electron reduction of molecular oxygen using as electron donor NADPH. The catalytic component of this complex is a membrane bound flavocytochrome *b*, referred to as flavocytochrome *b558* (cyt *b558*) which consists of a heterodimer of two non-covalently linked glycosylated proteins $p22^{phox}$ and $gp91^{phox}$. The biochemical studies indicate that in addition to the two hemes, cyt*b558* contains a functional FAD prosthetic group. There are, at the moment, neither direct structural data of cyt*b558*, nor on the way it binds its redox components. Various studies allowed to propose structural models but which remain still hypothetical. The limiting factor for functional and structural studies is the lack of sufficient amounts of the cyt*b558* in stable, pure and homogenous form. Therefore, we are focusing our efforts in producing the membrane heterodimer by designing an engineered efficient membrane protein expression tool. Thus, we have made attempts to express the cyt*b558* in an *Escherichia coli* system but the eukaryotic *Pichia pastoris* expression system leads to more successful results.

doi:10.1016/j.bbabio.2008.05.233

S10.8 Remodelling of mitochondrial function by the p13 protein of HTLV-1: Effects on reactive oxygen species and cell death

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In previous studies we showed that the p13 protein coded by human T-cell Leukemia/Lymphoma virus type-1 (HTLV-1) is targeted to mitochondria, affects cell turnover in vitro and exerts antitumor effects in experimental transformation models. The present study was aimed at understanding the mechanism of p13 function. Assays employing full-length synthetic p13 and isolated mitochondria showed that p13 triggers an inward K^+ current, resulting in depolarization, increased respiratory chain activity and accumulation of reactive oxygen species (ROS). Similar effects were induced by the K^+ ionophore valinomycin, while the protomophore FCCP reduced ROS production, suggesting that depolarization induced by K^+ vs. H^+ currents has opposite effects on ROS. We next studied the effects of p13 expression on reactive oxygen species production in HeLa and Jurkat T-cells. Results demonstrated that p13-expressing cells exhibit increased ROS levels and cell death. Interestingly, these effects ensued when cells were subjected to glucose deprivation, and were abrogated by treatment with ROS scavengers. Taken together, these findings indicate that by remodelling mitochondrial function, p13 may control mitochondrial ROS production and cell turnover under conditions of metabolic stress.

doi:10.1016/j.bbabio.2008.05.234

S10.9 Flavohemoproteins as potential targets of antibiotics

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The compound miconazole is the most widely used antimycotics. Imidazoles such as miconazole, econazole, ketoconazole and clotrimazole have been recently proposed to coordinate selectively bacterial flavohemoproteins thus inhibiting their oxyde nitric dioxygenases (NOD) activity. This enzymatic conversion thus protects bacteria from the toxic NO and from other damaging NO-derived

reactive nitrogen species produced by NO-release immune cells. The aim of this work is to further unravel the mode of action of miconazole on microorganism hemoprotein. Inhibitors that target flavohemoproteins are attractive candidates for antibiotic development. Spectroscopic analysis of the oxidized or reduced flavohemoprotein from *Ralstonia eutrophus* (FHP) in the presence of different antibiotics have been done. Addition of Miconazole and econazole and other antimicrobial substances from plants and algae caused spectroscopical change to FHP indicating heme coordination. To identify protein–drug interactions that contribute to binding specificity and affinity, we performed co-crystallization trials of FHP in the presence of miconazole or econazole. We have obtained crystals of FHP in complex with miconazole and econazole. X-ray diffraction experiments of these crystals is conducted in order to determine the crystallographic structure of the antibiotic–protein complex.

doi:10.1016/j.bbabio.2008.05.235

S10.10 Discrepancy between effects of nitroglycerin and nitric oxide on mitochondrial respiration

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Nitric oxide (NO) is known to inhibit mitochondrial respiration preferentially by binding to cytochrome oxidase. Such situation is expected in sepsis, which is accompanied by induction of inducible NO-synthase. Nitroglycerin (NG) is a widely used drug, which is believed to exert its biological activity through release of NO. This study aimed at comparison of effects NO and nitroglycerin on mitochondrial respiration and clarifying whether illumination at specific wavelengths recovers mitochondrial respiration inhibited by either NO or NG. NO fully inhibited respiration of liver mitochondria at concentrations occurring under septic shock. The respiration was completely restored by illumination at the wavelength of 430 nm while longer wavelengths were less effective. NG inhibited mitochondrial respiration though the efficiency of GTN was lower compared to NO concentrations observed in sepsis models. However, NG inhibition was absolutely insensitive to illumination regardless of wavelength used. Our data show that visible light of short wavelengths efficiently facilitates the recovery of mitochondria inhibited by NO-gas at the levels generated under septic conditions. The inhibition of mitochondrial respiration by NG is not sensitive to the visible light, suggesting another than NO-gas mediated mechanism of inhibition.

doi:10.1016/j.bbabio.2008.05.236

S10.11 Bioenergetic regulation of nitric oxide production in rat mitochondria

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Not only heart mitochondrial membranes (2.08 ± 0.08 nmol/min. mg protein), but also heart coupled mitochondria, exhibit an enzymatic production of NO. MtNOS activity is 40% lower in state 3 than in state 4, and shows an exponential dependence on membrane potential. The aim of this work was to further characterize mtNOS activity regulation by the redox state of the respiratory chain and membrane potential. The generation of NO (nmol/min mg protein) by heart submitochondrial particles resulted 0.45 ± 0.02. This value was enhanced up to 0.81 ± 0.09 when mtNOS activity was assessed in the presence of succinate and ATP. The addition of rotenone inhibited by 50% this reversed electron transfer-supported mtNOS activity. Besides, the ability of mtNOS to modulate O_2 uptake and H_2O_2 production, is termed mtNOS functional activity. Supplementation of state 3 mitochondria with L-arginine decreased respiration rates by 15–20%, while addition of L-NAME increased O_2 consumption by 10%. The addition of L-arginine enhanced state 4 H_2O_2 production by 14–21%, whereas supplementation with L-NAME declined H_2O_2 generation by 7–9%. Interestingly, these effects were observed in coupled mitochondria, but not in mitochondrial membranes. We conclude through direct and indirect evidence, that mtNOS activity is regulated by membrane potential; and that respiratory chain electron flow modulates NO production; in agreement with the reported physical interaction of mtNOS and respiratory chain components.

doi:10.1016/j.bbabio.2008.05.237

S10.12 Kinetic model of nitric oxide inhibition of cellular respiration in intact cells

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A kinetic model of nitric oxide (NO) inhibition of cellular respiration was developed in HEK 293 cells expressing the inducible isoform of the nitric oxide synthase (iNOS). Endogenous NO production (ISO-NOP, WPI), O_2 concentration and O_2 flux (OROBOROS Oxygraph-2k) were simultaneously recorded in an extended range of O_2 concentrations. Both competitive reversible binding of NO to reduced cytochrome *c* oxidase (CCO) and uncompetitive binding to oxidized CCO were taken into account. Data analysis, by means of standard least squares non linear minimization routines (Matlab, the MathWorks inc., South Natick, MA, USA), showed that the best fit to the experimental data requires the affinity of CCO for O_2 to be modulated by NO bound to the enzyme, such that the species with NO bound to the uncompetitive site has higher K_m than uninhibited CCO, consistent with the inhibitory activity of NO. Our scheme implies that the oxidized CCO derivative bearing NO bound to Cu_B consumes oxygen, albeit with poor efficiency, in a cycle that presumably releases nitrite or nitrate. The model has predictive value and integrates the complex chemistry of the enzyme and physiological adaptations of the cell. Interestingly, addition of NO scavengers reveals that NO has an activation effect in cells, which partially compensates for inhibition.

doi:10.1016/j.bbabio.2008.05.238

S10.13 Toxicity of parabens in testis mitochondria; a possible role on male infertility

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Parabens are widely used as preservatives in many foods, cosmetics, toiletries, and pharmaceuticals due to their relatively