These observations add to the list of AP-induced phenomena in plants, thus emphasizing the significance of AP as a multifunctional signal. We discuss the transient changes in pH-banding, effective quantum yield and non-photochemical quenching in relation to alterations in intracellular Ca^{2+} and H^+ concentrations during and after AP.

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S8.13 Effect of α-overexpression of F_1F_o-ATP synthase on iron-overloaded heart

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The aim of our study was to examine the effect of overexpression of α subunit of F_1F_o-ATP synthase on iron uptake of mitochondria and cardiac myocytes under iron overload. We did a transfection of α subunit gene on the primary cardiac myocytes of iron-overloaded rats. In α-overexpressed cells, mitochondrial ROS was reduced (4.14± 0.23 vs. 1.77±0.03 μM/mg; non- vs. overexpressed) and free iron as well, which was reduced to 40% (0.98±0.04 vs. 0.39±0.05 μmol/mg). The mitochondrial ATPase activity increased 2-fold (0.27±0.07 vs. 0.54± 0.05 mM/mg/min) and mitochondrial ATP was lowered to 29% (1.27± 0.10 vs. 0.37±0.03 $^{E-07}$ mol/mg) in α-overexpressed cells compared to control. In α-overexpressed cells of iron-overloaded heart, mitochondrial LIP was increased (2.65±0.42 vs. 3.67± 0.45 μmol/mg). Mitochondrial ATP was slightly reduced (0.35±0.10 vs. 0.25±0.03 $^{E-07}$ mol/mg) although mitochondrial ROS (7.39±0.23 vs. 16.69 ± 0.54) and ATPase activity (0.49±0.10 vs. 1.06±0.01) were expanded over 2-fold. Mitochondrial membrane potential was significantly augmented (100±15 vs. 270±39% by rhodamine 123 staining). We found declined cellular LIP level (13.7±0.36 vs. 7.43± 0.82 μmol/mg) but ugmented ATP amount over 2.4-fold (0.30±0.50 vs. 0.70±0.03 $^{E-07}$ mol/mg). Importantly, we observed that iron-overloaded cells have higher viability with α-overexpression than without. And, signals of apoptotic cell death were considerably declined in the presence of iron. Based on these data, we suggest that overexpressed α subunit contributes to the viability in iron-overloaded heart.

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S8.14 Low power long wavelength laser irradiation effects on human mononuclear cell mitochondrial membrane potential

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The objective of this study was to demonstrate therapeutic soft laser light effects on the mitochondrial membrane electrical potential in human peripheral blood mononuclear cell subpopulations in various culture and irradiation conditions. Furthermore we observed microenvironment dependent cross-talk between separately irradiated adherent and non-adherent mononuclear cells grown in co-culture. Quantitative analysis of JC-1 red/green fluorescence signals, gathered on surface antigen labeled single cells by flow cytometry, allowed us to disclose changes in both the relative sizes of adherent/non-adherent cell subpopulations with preponderently highly/weakly polarized mitochondrial membranes, and in the average mitochondrial membrane potentials of these subpopulations. The changes induced in the mitochondrial membrane state by the 680 nm far-red and 830 nm infrared laser lights were single and total dose, wavelength, irradiation regime, and cell-state dependent. Metabolic modulation of laser effects was evident. As a rule energy/nutrient restricted cells with altered mitochondrial membranes were more sensitive to soft laser irradiation than the non-injured controls. Irradiation of adherent cells caused more substantial changes in the mitochondrial membrane state. Cross-talk between irradiated and non-irradiated cells in co-culture was evident in the presence of growth factors.

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S8.15 Liver metabolic fluxes in response to high fat diet

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Aim: Determining adaptations of metabolic fluxes in liver with high fat diet (HF).
Methods: Glucose, lactate and pyruvate production by perifused hepatocytes isolated from rats fed either a standard or HF diet.
Results: HF increased gluconeogenesis from glycerol in the presence of octanoate (+30%) but decreased it in the absence of fatty acid. This effect was associated with an increase of glycerol metabolism without effect on glycolysis. In both conditions, cytosol was more oxidized whereas mitochondrial compartment was more reduced. Cellular and mitochondrial oxidative capacities were reduced by HF (−40%). Glycerol metabolism requires a stoichiometric utilization of ATP and NAD^+. Therefore depending on the redox condition, control of the pathway is either on the dehydrogenase step, (at low rate of glycerol metabolism), or on the phosphorylation step (high rate of metabolism). Hence, the lower glycerol metabolism observed with HF at high flux is due to diminished oxidative phosphorylation capacity and to an inability to maintain ATP. By contrast, when flux through the pathway is reduced by high redox pressure of fatty acid metabolism, the ability of HF animals to maintain an oxidized cytosolic compartment allows then to metabolize more glycerol. This feature probably results from a higher rate of NADH oxidation via the mitochondrial glycerophosphate dehydrogenase which could be an adaptation to HF for compensating the decrease in oxidative phosphorylation capacity.

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S8.16 Mitochondrial adjustment to energy demand when cell growth slows down

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During cellular proliferation on non-fermentable carbon source, mitochondrial activity must meet energy demand. Previous work in

yeast has shown that, during aerobic growth, cAMP pathway contributes to the adjustment of the mitochondria to the energy demand in order to maintain a constant growth yield by modulating the amount of mitochondria when cell growth slows down. The aim of this study was to determine the origin of mitochondrial decrease (energy demand decrease by drop cell proliferation, inhibition of mitochondrial biogenesis). We take cells in proliferation state and we arrest proliferation by transferring cells into a resting medium. Hence, we have the possibility to artificially increase energy demand. Our first data without energy demand increase show that mitochondrial regulation first involves a modification of the mitochondrial steady state respiration (as shown by oxygen consumption) and then a pathway which requires a new cytosolic protein synthesis (as shown by experiments in the presence of cycloheximide). Using various yeast strains, we show that the adjustment is identical regardless of the strain. Concurrently, analyses of mitochondrial enzymatic activities, western-blot, electronic and fluorescence microscopy show that there is no modification of mitochondrial amount. This study points out to a process of mitochondrial amount adjustment, during growth, which needs cell proliferation in order to drop mitochondrial amount.

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S8.17 siRNA knock-down of creatine kinase in rat primary myotube culture

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The aim of the study was to establish a method for transfection of rat primary myotubes with siRNA.

Myoblasts were isolated from the hind legs of newborn Wistar rats and grown on matrigel® coated culture dishes. The cells were considered differentiated on day 11–15 of culture (J. Biol. Chem. (2002) 277, 4831). The fluorescent positive transfection control siGLO® (Dharmacon) or siRNA molecules directed against three isoforms of creatine kinase (CKM, CKB and sMtCK) were transfected using three different transfection agents: Oligofectamine® (Invitrogen), X-tremeGENE® (Roche) and Dharmafect4® (Dharmacon). We found that successful transfection was only obtained with Dharmafect4®, that the optimal concentration of siRNA was 100 nM and that the most favourable time point for transfection was on day 7 of our protocol. The CKB messenger was undetectable, whereas we were able to decrease the mRNA levels of the sMtCK and CKM isoform of creatine kinase by app. 65%. The corresponding levels of creatine kinase activity were only reduced app. 33% with the CKM siRNA, suggesting a half-life of the enzyme exceeding the duration of our experiment. The creatine kinase activity was not reduced by the sMtCK siRNA, indicating that CKM is the predominant isoform in our culture system.

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S8.19 Interaction of pyruvate and fatty acid oxidation in primary cultures of rat myotubes

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Aim: To study the interaction of pyruvate, oleate and eicosapentenoic acid (EPA) oxidation in primary cultures of rat myotubes (J. Biol. Chem. (2002) 277, 4831). Pyruvate oxidation had an apparent K_m value of 1 mM. Fatty acid oxidation increased linearly with the concentration of fatty acid. Pyruvate oxidation was inhibited 34±7% by 0.1 mM oleate and 46±6% by 0.2 mM oleate at 0.15 mM pyruvate. In contrast, 0.15 mM pyruvate had no effect on oleate or EPA oxidation. 3-Hydroxybutyrate (1.5 mM), presumably causing a more reduced mitochondrial matrix NAD-redox state, inhibited pyruvate oxidation by 60%, but oleate oxidation by only 15%. Reduced cytosolic NAD-redox state (by 1.5 mM lactate) resulted in 70% inhibition of oleate oxidation. Experiments with isolated skeletal muscle mitochondria respiring at 50% of state 3 or at state 3 showed a ca. 40% inhibition of pyruvate oxidation by palmitoyl–carnitine, but only 0–20% inhibition of palmitoyl–carnitine oxidation by pyruvate. Thus, pyruvate oxidation is more prone to inhibition by fatty acid oxidation than *vice versa*. It appears that both the cytosolic and the mitochondrial NAD-redox states are involved in the regulation of substrate choice by muscle cells.

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S8.20 The mechanisms leading to the Crabtree effect in fermenting yeast

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The Crabtree effect is defined as the glucose-induced repression of respiratory flux; its triggering mechanisms are still unknown. *Saccharomyces cerevisae* exhibits a Crabtree effect during fermentation. In these conditions a decrease of cytoplasmic phosphate levels and an increase of NAD^+ have been observed. At the same time, glycolysis hexoses phosphates accumulate in the cytoplasm, particularly fructose 1,6-biphosphate (F16bP). In order to explain the Crabtree effect, we analyzed the interactions between F16bP, phosphate and NAD^+. In isolated mitochondria and in permeabilized spheroplasts F16bP inhibited the respiratory flux. The levels required for this inhibition were similar to those observed in the cytoplasm of yeast cells at the beginning of fermentation. In isolated mitochondria, reduction of the NADH steady-state levels using a NADH-regenerating system lead to a decrease of the rate of oxygen consumption in yeast mitochondria, which were further inhibited in the presence of F16bP. By contrast, decreasing phosphate levels increased respiratory flux. However, this effect was fully counteracted by F16bP. During fermentation in yeast, the decrease in the NADH/NAD^+ ratio, plus the increased cytoplasmic F16bP levels contribute to the Crabtree effect induction.

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S8.21 Rapid effect of 3,5-diiodo-L-thyronine on mitochondrial fatty acid oxidation and thermogenesis in skeletal muscle

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