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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