

that seem to indicate two quinone pools that are involved in the electron right of way. Indeed, the more reduced pool would be associated to the electron right of way for the external dehydrogenases whereas the less reduced pool would be associated to the electron right of way for the internal dehydrogenases.

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#### 17L.4 Systems biology and bioenergetics: Structure–function relationships in feedback regulation of energy fluxes *in vivo* Mitochondrial interactosome

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The aim of this review is to analyze the results of experimental research of mechanisms of regulation of mitochondrial respiration in cardiac and skeletal muscle cells *in vivo* obtained by using the permeabilized cell technique. Such an analysis in the framework of Molecular Systems Bioenergetics shows that the mechanisms of regulation of energy fluxes depend on structural organization of the cells and interaction of mitochondria with cytoskeletal elements. Two types of cells of cardiac phenotype with very different structure were analyzed: adult cardiomyocytes and continuously dividing cancerous HL-1 cells. In cardiomyocytes mitochondria are arranged very regularly, show rapid configuration changes of inner membrane but no fusion or fission, and diffusion of ADP and ATP is restricted mostly at the level of mitochondrial outer membrane due to interaction of heterodimeric tubulin with voltage dependent anion channel, VDAC. VDAC with associated tubulin forms a supercomplex, mitochondrial interactosome, with mitochondrial creatine kinase, MtCK, which is structurally and functionally coupled to ATP synthasome. Due to selectively limited permeability of VDAC for adenine nucleotides, mitochondrial respiration rate depends almost linearly upon the changes of cytoplasmic ADP concentration in their physiological range. Functional coupling of MtCK with ATP synthasome amplifies this signal by recycling adenine nucleotides in mitochondria coupled to effective phosphocreatine synthesis. In cancerous HL-1 cells this complex is significantly modified: tubulin is replaced by hexokinase and MtCK is lacking, resulting in direct utilization of mitochondrial ATP for glycolytic lactate production and in this way contributing in the mechanism of the Warburg effect. Systemic analysis of changes in the integrated system of energy metabolism is also helpful for better understanding of pathogenesis of many other diseases.

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#### 17P.1 Cyclophilin D decreases ATP hydrolysis and synthesis rates of the F<sub>0</sub>F<sub>1</sub>-ATP synthase, unaffected ADP–ATP flux rates in intact mitochondria

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Cyclophilin D was recently shown to bind to F<sub>0</sub>F<sub>1</sub>-ATP synthase, modulating the activity of this complex in submitochondrial particles

and permeabilized mitochondria (Giorgio V et al., 2009, J. Biol. Chem. 284: 33982–33988). Binding of cyclophilin D decreased both the ATP synthesis and hydrolysis rates by the F<sub>0</sub>F<sub>1</sub>-ATP synthase. Here, we reaffirm these findings by showing that the absence of cyclophilin D or the presence of cyclosporin A increased respiration rates, and decreased the extent of uncoupler-induced depolarization in ATP-energized intact mitochondria, isolated from mouse liver. This implied a boosted proton pumping by the ATPase in both synthesis and hydrolysis mode. However, the modulation of F<sub>0</sub>F<sub>1</sub>-ATP synthase by cyclophilin D did not translate to an increase in ATP efflux in energized mitochondria, nor to an increase in ATP influx from deenergized mitochondria, reflecting the imposing role of the ANT in ADP–ATP flux processes of intact mitochondria. We conclude that ablation of *ppif* gene or inhibition of cyclophilin D binding to F<sub>0</sub>F<sub>1</sub>-ATP synthase by cyclosporin A will i) affect only matrix adenine nucleotide levels for as long as the inner mitochondrial membrane remains intact, and ii) will render only permeabilized mitochondria as enhanced ATP consumers. Furthermore, provided that energization decreases the probability of pore opening, we propose that abolition of cyclophilin D or its binding on the F<sub>0</sub>F<sub>1</sub>-ATP synthase, may delay permeability transition pore by simply inducing an accelerated flux of ATP hydrolysis, thereby increasing protonmotive force of compromised mitochondria.

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#### 17P.2 Metabolic control analysis of integrated energy metabolism in permeabilized cardiomyocytes

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Metabolic control analysis (MCA) shows quantitatively the degree of control that a given enzyme or enzyme complex exerts on the flux of the metabolites, it gives us knowledge whether the control of the pathway is shared between the different enzymes or transporters in the pathway. Until now the tool of MCA in Bioenergetics is used mostly for the analysis of respiration of isolated mitochondria. Our recent results have shown selective control of permeability of mitochondrial outer membrane voltage-dependent anion channel (VDAC) by heterodimeric tubulin in the permeabilized cardiac cells *in situ*. Under these conditions, diffusion of ADP and ATP is limited and the energy liberated in oxidative phosphorylation is carried from mitochondria into cytoplasm by phosphocreatine. The mitochondrial creatine kinase, MtCK within the supercomplex of ATP synthasome–MtCK–VDAC–tubulin, or mitochondrial interactosome in heart mitochondria is therefore central for the control of respiration. The aim of the present study was to use the method of metabolic control analysis for quantitative description of the role of mitochondrial interactosome and respiratory chain components in the control of energy fluxes in permeabilized cardiomyocytes *in situ*. In this experimental model we measured the energy flux as the steady state rate of oxygen consumption in the interactosome in the presence of creatine, 20 mM. The components of the integrated pathway of free energy conversion studied were the respiratory complexes, the MtCK complex, Pi transporter and ATP/ADP transporter. For the measurements of flux control coefficients of each component permeabilized cardiomyocytes were titrated with the specific inhibitors for a stepwise decrease of activity of each complex, while the changes in the oxygen consumption rate was measured. The protocol used for the measurements included the pyruvate kinase (PK) – phosphoenol pyruvate (PEP) system to assure that only the ADP produced in mitochondria is taken