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

V. Saks[a,b], R. Guzun[a], N. Timohhina[b], K. Tepp[b], M. Varikmaa[b], C. Monge[a], N. Beraud[a], T. Kaambre[b], A. Kuznetsov[c], L. Kadaja[c], E. Margus[d], E. Seppet[d]
[a]*INSERM U884, Joseph Fourier University, Grenoble, France*
[b]*National Institute of Chemical Physics and Biophysics, Tallinn, Estonia*
[c]*Innsbruck Medical University, Austria*
[d]*University of Tartu, Tartu, Estonia*
E-mail: Valdur.Saks@ujfrenoble.fr

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_OF_1-ATP synthase, unaffecting ADP–ATP flux rates in intact mitochondria

Christos Chinopoulos[a,b], Steven F. Zhang[a], Anatoly A. Starkov[a]
[a]*Weill Medical College Cornell University, New York, NY, 10021, USA*
[b]*Department of Medical Biochemistry, Semmelweis University, Budapest, 1094, Hungary*
E-mail: cchinopoulos@yahoo.com

Cyclophilin D was recently shown to bind to F_OF_1-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_OF_1-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_OF_1-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_OF_1-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_OF_1-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

Kersti Tepp[a], Natalja Timohhina[a], Vladimir Chekulayev[a], Igor Shevchuk[a], Tuuli Kaambre[a], Valdur Saks[a,b]
[a]*Laboratory of Bioenergetics, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia*
[b]*Laboratory of Fundamental and Applied Bioenergetics, INSERM U884, Joseph Fourier University, Grenoble, France*
E-mail: tuuli.kaambre@kbfi.ee

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, MiCK 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

into account. All the inhibitors used were considered irreversible and noncompetitive under these conditions. The inhibitors used were rotenone for Complex I of the respiratory chain, antimycin A for Complex III, cyanide for Complex IV, carboxyatractyloside for ATP/ADP transporter, oligomycin for ATP synthase and mersalyl for Pi transporter. For mitochondrial CK inhibition dinitrofluorobenzene was used. In this way, flux control coefficients were found for all components described.

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17P.3 Respiratory coupling in the mitochondrial electron transport chain in RAW 264.7 cells

Roger Springett, Michelle Abajian, Maureen Ripple
Department of Radiology, Dartmouth Medical School, USA
E-mail: RSpringett@Dartmouth.edu

Respiratory coupling, defined as the ability of mitochondria to increase oxygen consumption (VO_2) in the face of increased cytosolic ATP demand, was studied in intact RAW cells using oligomycin to model low ATP demand and FCCP to model increases in ATP demand. VO_2 was measured using respirometry, the oxidation changes of the cytochromes of the electron transport chain were monitored using multi-wavelength spectroscopy and NAD(P)H oxidation changes were measured using fluorescence spectroscopy. The mitochondrial membrane potential ($\Delta\psi$) and redox potential (E_h) of the ubiquinone pool (UQ) were calculated from the redox poise of the b_H and b_L centers of the bc_1 complex using a model that takes into account their redox cooperativity. After inhibition with oligomycin, the VO_2 response to FCCP could be split into two regions. At low concentrations of FCCP (0–150 nM) VO_2 increased 11-fold with increasing FCCP (positive respiratory coupling) whereas at high concentrations of FCCP (200–500 nM), VO_2 decreased with increasing FCCP (negative respiratory coupling). Over the positive respiratory coupling range, $\Delta\psi$ decreased from 175 ± 3 to 135 ± 10 mV (mean $\pm$ SD, $n = 6$). The heme *a* center of cytochrome oxidase reduced from 90 ± 2 to $84 \pm 2\%$ oxidized. Contrary to the results from isolated mitochondria, cytochrome *c* initially reduced from $76 \pm 2\%$ to $72 \pm 2\%$ oxidized (0–100 nM) and then reoxidized back to $76 \pm 2\%$ oxidized at 150 nM FCCP. The oxidation state of heme b_H was almost independent of FCCP at about 70% oxidized whereas b_L oxidized from $32 \pm 2\%$ to $61 \pm 7\%$ oxidized. However, the calculated E_h of b_H increased from 22 ± 5 mV to 45 ± 6 mV due to the redox cooperativity between b_H and b_L and the E_h of UQ increased from 66 ± 5 mV to 74 ± 5 mV. Preliminary results showed that NADH varied from almost fully reduced to almost fully oxidized. All the cytochromes and NADH became highly oxidized in the negative respiratory coupling range. The results suggest that cytochrome oxidase is almost entirely responsible for respiratory coupling in RAW cells and that it responds to the decline in $\Delta\psi$ by increasing its turnover by 11-fold as $\Delta\psi$ decreases from 175 to 135 mV at a constant cytochrome *c* E_h of 290 mV. The E_h of UQ and NADH increase due to the declining energy requirement of the proton pumps in the bc_1 complex and complex I respectively.

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17P.4 Increased expression of the cardiac ubiquitin ligase MuRF1 alters mitochondrial bioenergetic capacity *in vivo*

Makhosazane Zungu[a], Jessica E. Rodriguez[b], Luge Li[b], Monte S. Willis[b,c]
[a]*University of KwaZulu Natal, Discipline of Human Physiology, Durban, South Africa*
[b]*University of North Carolina, Department of Pathology and Laboratory Medicine, Chapel Hill, NC USA*
[c]*University of North Carolina, McAllister Heart Institute, Chapel Hill, USA*
E-mail: zungumak@ukzn.ac.za

Muscle ring finger protein 1 (MuRF1) is a muscle-specific RING-finger-dependent ubiquitin ligase that regulates the development of cardiac hypertrophy, PPARα-mediated fatty acid metabolism, and creatine kinase activity. We have identified these mechanisms using a model of constitutive over-expression of cardiac MuRF1 (MuRF1 Tg^+) and ablation of MuRF1 (*MuRF1*$^-$). At baseline, MuRF1 Tg^+ hearts exhibit a 15% reduction in fractional shortening and a 20% reduction in LV wall thickness compared to strain-matched wild type controls. *MuRF1*$^-$ hearts do not have a discernable phenotype from wild type controls. Microarray analysis of the MuRF1 Tg^+ hearts revealed that genes involved in mitochondrial oxidative phosphorylation are significantly decreased, including ATP50, NDUFB8, NDUFS6, NDFS7, NDUFV2, and SDHA. Here, we investigated the effects of MuRF1 Tg^+ and *MuRF1*$^-$ on cardiac mitochondrial bioenergetic capacity. Cardiac mitochondria from MuRF1 Tg^+ and *MuRF1*$^-$ were isolated by differential centrifugation and respiration parameters were measured using a Clarke-type oxygen electrode employing a combination of malate (M), pyruvate (P), palmitoyl-l-carnitine (CP) and succinate as oxidative substrates. Oligomycin-insensitive basal proton leak was augmented in the MuRF1 Tg^+ cardiac mitochondria by $40 \pm 2.4\%$ ($n = 5$; $p < 0.05$ vs. wild-type control) in the presence of M + P. The respiratory control index (RCI), a marker of mitochondrial viability, was increased in the *MuRF1*$^-$ mice by $48 \pm 4.5\%$ ($n = 5$; $p < 0.05$ vs. wild-type control) when succinate was employed as an oxidative substrate. To determine how MuRF1 expression might affect mitochondrial number, we assayed the mitochondrial genes Cytb1, C01, Ndl by RT-PCR. We found that mitochondrial DNA content in the MuRF1 Tg^+ and *MuRF1*$^-$ hearts were not significantly different compared to the wild-type controls, respectively. Our data demonstrate that increased cardiac MuRF1 expression impairs mitochondrial oxidative phosphorylation, possibly as a result of increased proton leak. The changes observed in oxidative phosphorylation genes in the MuRF1 Tg^+ hearts suggest transcription as a more specific regulation of oxidative phosphorylation and not mitochondrial number.

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