



Review

Regulation of respiration in muscle cells in vivo by VDAC through interaction with the cytoskeleton and MtCK within Mitochondrial Interactosome[☆]

Rita Guzun^{a,*}, Marcela Gonzalez-Granillo^a, Minna Karu-Varikmaa^b, Alexei Grichine^c, Yves Usson^c, Tuuli Kaambre^b, Karen Guerrero-Roesch^a, Andrey Kuznetsov^d, Uwe Schlattner^a, Valdur Saks^{a,b}

^a INSERM U1055, Laboratory of Fundamental and Applied Bioenergetics, Joseph Fourier University, Grenoble, France

^b Laboratory of Bioenergetics, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia

^c Life science imaging – in vitro platform, IAB CRI U823 Inserm/Joseph Fourier University, France

^d Cardiac Surgery Research Laboratory, Department of Heart Surgery, Innsbruck Medical University, Innsbruck, Austria

ARTICLE INFO

Article history:

Received 1 November 2011

Received in revised form 26 December 2011

Accepted 29 December 2011

Available online 4 January 2012

Keywords:

Mitochondria

Voltage-dependent anion channel

Cytoskeleton

Tubulin

Phosphotransfer network

Creatine kinase system

ABSTRACT

This review describes the recent experimental data on the importance of the VDAC–cytoskeleton interactions in determining the mechanisms of energy and metabolite transfer between mitochondria and cytoplasm in cardiac cells. In the intermembrane space mitochondrial creatine kinase connects VDAC with adenine nucleotide translocase and ATP synthase complex, on the cytoplasmic side VDAC is linked to cytoskeletal proteins. Applying immunofluorescent imaging and Western blot analysis we have shown that β 2-tubulin coexpressed with mitochondria is highly important for cardiac muscle cells mitochondrial metabolism. Since it has been shown by Rostovtseva et al. that $\alpha\beta$ -heterodimer of tubulin binds to VDAC and decreases its permeability, we suppose that the β -tubulin subunit is bound on the cytoplasmic side and α -tubulin C-terminal tail is inserted into VDAC. Other cytoskeletal proteins, such as plectin and desmin may be involved in this process. The result of VDAC–cytoskeletal interactions is selective restriction of the channel permeability for adenine nucleotides but not for creatine or phosphocreatine that favors energy transfer via the phosphocreatine pathway. In some types of cancer cells these interactions are altered favoring the hexokinase binding and thus explaining the Warburg effect of increased glycolytic lactate production in these cells. This article is part of a Special Issue entitled: VDAC structure, function, and regulation of mitochondrial metabolism.

© 2012 Elsevier B.V. All rights reserved.

Contents

1. Structural and functional organization of cardiac muscle cells' energy metabolism in the Intracellular Energy Units	1545
2. The β 2-tubulin – “X” factor in adult cardiac muscle cells	1546
3. The influence of VDAC–tubulin interaction on mitochondrial functioning	1547
4. Energy fluxes between different cellular compartments	1548
5. Metabolic control analysis within Mitochondrial Interactosome	1549
6. The VDAC selective permeability in physiological and pathological states	1552
Acknowledgements	1552
References	1552

1. Structural and functional organization of cardiac muscle cells' energy metabolism in the Intracellular Energy Units

The Frank–Starling's law describes the ability of heart to change the force of contraction and stroke volume in response to changes in the end-diastolic volume [1–3]. The metabolic aspect of this law is expressed by linear dependence between the increase of left ventricular end-diastolic volume and the increase of respiration rates

[☆] This article is part of a Special Issue entitled: VDAC structure, function, and regulation of mitochondrial metabolism.

* Corresponding author at: Laboratory of Bioenergetics, Joseph Fourier University, 2280, Rue de la Piscine, BP53X38041, Grenoble Cedex 9, France.

E-mail address: rita.guzun@gmail.com (R. Guzun).

under conditions of metabolic stability. The metabolic stability is seen as the apparent invariability of intracellular concentration of ATP and phosphocreatine (PCr) in spite of the variable workloads and corresponding rates of ATP oxidative synthesis and myofibrillar hydrolysis [4,5]. Under conditions of total ischemia the PCr concentration falls rapidly and heart contraction ceases, but ATP concentration stays almost stable decreasing only by 10% at the end of the first minute of ischemia [6]. Two complementary mechanisms were proposed in order to explain this remarkable metabolic stability, notably adenine metabolite compartmentalization and the transfer of high-energy phosphoryls between micro-compartments associated to a very effective communication by intracellular signals from myofibrils to mitochondria. These mechanisms depend both on the heterogeneity of the intracellular environment causing restrictions for metabolites diffusion, as well as upon the specific functional characteristics of the heart resulting from its high energy needs. The mitochondria of cardiac muscle cells are highly organized following a “crystal-like” pattern [7,8]. Each sarcomere (site of ATP use) has its own corresponding mitochondrion (site of ATP synthesis) which together with the phosphotransfer system and the feed-back metabolic signaling creates the Intracellular Energy Unit (ICEU) [9–11]. The main ICEUs phospho-transfer circuit is represented by a system of compartmentalized isoforms of creatine kinase (CK). The proper functioning of ICEU is based on the regulation of mitochondrial ATP synthesis by intracellular energy consuming processes which adjust energy extraction from nutrients to energy used to realize cellular work [11]. The central structural and functional entity involved into the regulation of energy fluxes leaving and metabolic signals entering mitochondria is so named Mitochondrial Interactosome (MI) [12]. The MI is a trans-membrane supercomplex formed of ATP synthasome, mitochondrial creatine kinase (MtCK) and voltage-dependent anion channel (VDAC). The etymology of interactosome comes from ATP synthasome, the name of its main structural element introduced by Pedersen [13]. The ATP synthasome is formed by ATP synthase, adenine nucleotide translocase (ANT) and inorganic phosphate carrier. In this review we will focus on the role of VDAC as a door-keeper regulating mitochondrial energy and metabolic fluxes, as a result of its interaction with both mitochondrial and extramitochondrial intracellular proteins.

2. The β 2-tubulin – “X” factor in adult cardiac muscle cells

Kinetic analysis of the regulation of respiration has revealed that the apparent affinity for ADP of cardiac muscle cells and tissue homogenate increases many times after trypsin pre-treatment or mitochondria isolation. The apparent K_m for ADP was found to be about 300–500 μM for the heart tissue homogenate and isolated cardiomyocytes [14], while isolated heart mitochondria displayed the apparent K_m ADP equal to 8–12 μM (Fig. 1). The significant decrease of the high apparent K_m ADP after mild trypsin treatment of cardiomyocytes (Fig. 1) has led to the hypothesis according to which the restriction of ADP diffusion through mitochondrial outer membrane (MOM) could be modulated by the VDAC interaction with a cytoskeleton protein, named at that moment “X”-factor [14].

Years of experimental work performed by different laboratories have allowed to focus the research of certain cytoskeleton proteins such as, tubulin, microtubule-associated protein (MAP), desmin, and plectin [15–22]. As recent as 2008, Rostovtseva et al., have shown that dimeric $\alpha\beta$ -tubulin can bind to VDAC reconstituted into planar lipid membrane and decrease its diameter [23,24]. Authors supposed that one of tubulin’s negatively charged C-terminal tails (CTT) partially blocks the channel conductance by entering the VDAC pore and binding to the positively charged channel walls [25,26]. The tubulin binding to VDAC restricts significantly the availability of ADP for oxidative phosphorylation, the low apparent K_m ADP of isolated heart mitochondria increased from 10–20 μM up to $169 \pm 52 \mu\text{M}$ following

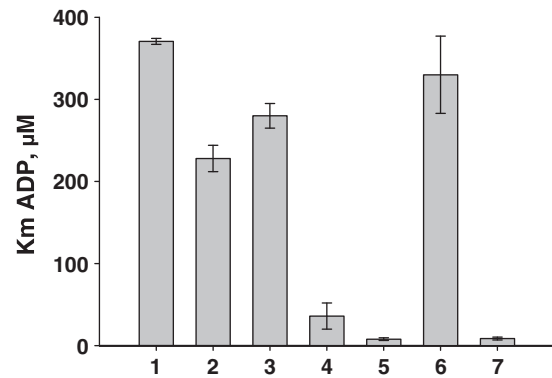


Fig. 1. Regulation of mitochondrial respiration by exogenous ADP estimated for: 1 – permeabilized cardiomyocytes; 2 – homogenate of tissue heart; 3 – ghost cardiomyocytes (the myosin was removed by the hyperosmotic cells pre-treatment using 0.8 M KCl); 4 – tissue heart homogenate after trypsin proteolysis; 5 – isolated heart mitochondria; 6 – isolated heart mitochondria after addition of 10 μM dimeric $\alpha\beta$ -tubulin; 7 – permeabilized NB HL-1 cells. High apparent K_m for ADP in permeabilized cardiomyocytes and cardiac muscle homogenate decreases after trypsin proteolysis showing the increase of mitochondrial outer membrane (MOM) permeability for ADP. Increase of MOM permeability is caused by the proteolytic remove of β II-tubulin and other linker proteins from VDAC’s protein-binding site. Isolated mitochondria and nonbeating (NB) HL-1 cells are characterized by very low K_m ADP and very high MOM permeability for ADP, respectively. This high permeability is caused by the absence of β II-tubulin (see Western blot [28]). Addition of the dimeric $\alpha\beta$ -tubulin to isolated mitochondria results in increased apparent K_m ADP (i.e. decreased availability of exogenous ADP for mitochondrial respiration).

the addition of 1 μM of dimeric $\alpha\beta$ -tubulin [27]. This results in the control of respiration through modification of VDAC permeability. Our studies of intracellular distribution of different β -tubulin isotypes in cardiac cells using immunocytochemistry revealed the role of the β 2-tubulin as one of the potential regulatory proteins of VDAC in adult rat cardiomyocytes [28,29]. Results shown in Fig. 2 clearly demonstrate the association of β 2-tubulin with heart mitochondria *in vivo*. We have shown that the distribution of β -tubulin isotypes differs significantly between cardiomyocytes and cancerous cardiac cells (NB HL-1 cells) which gives rise to the question how do the specific localization and functioning of β -tubulin isotypes contribute to the regulation of energy metabolism and contraction motion (shrinking and relaxing)? It is well established that the CTT of different α - and β -tubulin isotypes are not involved in the formation of intradimer surface during polymerization and have almost similar length (from 10 to 20 aminoacid residues) and electronegative charges (from about –7 to –9) [30]. Because of the structural similarities between CTT it is conceivable for the functional role of β -tubulin isotypes to be exchangeable and therefore gene knockdown or silencing of specific β -tubulin isotypes might prove unable to answer this question. However, it is important to mention that the complete genetic deletion of the tails is lethal for microorganism [31]. Furthermore, the role of α -tubulin isotypes for the regulation of mitochondrial metabolism is still uncovered. Studies of the intracellular distribution of α -tubulin isotypes could help to understand their function relative to VDAC and β 2-tubulin CTT binding to porin. We suppose that the CTT of α -tubulin slices into the channel as it is described by Rostovtseva [25,26] while that of β 2-tubulin binds to the VDAC binding site situated on the cytosolic face, according to the VDAC functional conformation proposed by Colombini (Fig. 3) [32,33]. Our assumption relies on the results of immunofluorescent analysis of β -tubulin distribution in cardiomyocytes. The easily reproducible fluorescent labeling of β 2-tubulin with antibody oriented against its CTT is probably possible only when the CTT epitope is outside of the VDAC channel (Fig. 3). It is interesting to mention that in cardiac cells treated with colchicine in order to depolymerize the microtubular network or remove myosin with a 0.8 M KCl buffer (ghost cells), tubulin seems to remain associated with mitochondria.

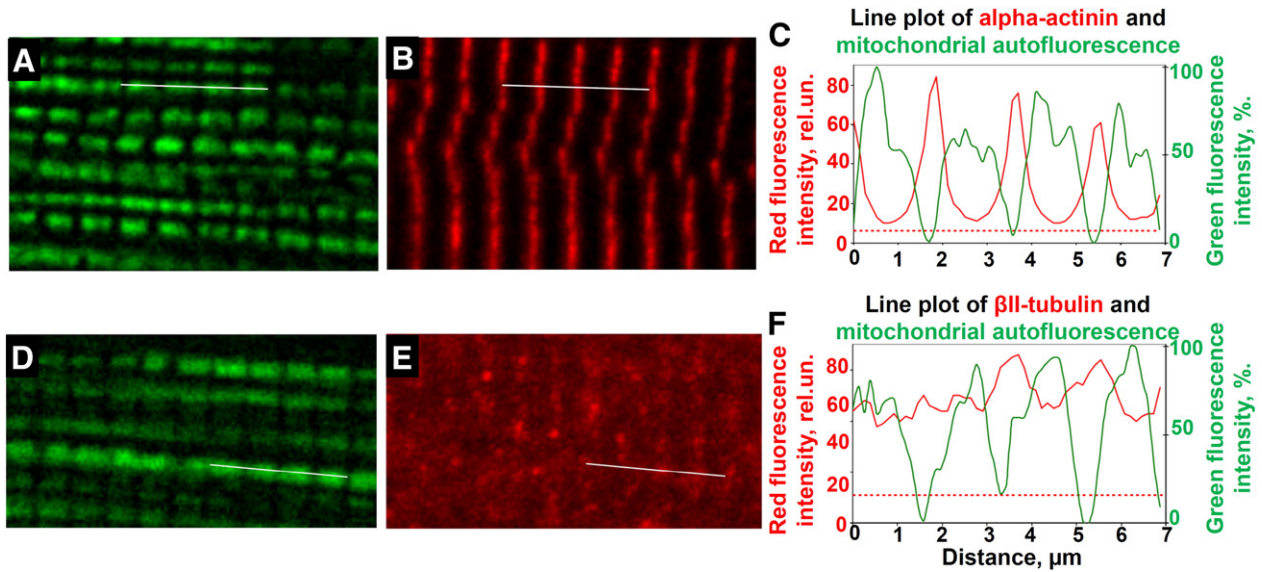


Fig. 2. Comparison of the intracellular distribution of β II-tubulin, α -actinin, a “Z-line label”, and mitochondria. A, D – confocal images of mitochondrial autofluorescence, green color. B – immunofluorescence labeling of α -actinin (primary antibody: rabbit anti- α -actinin (Abcam) at 1/100, anti-Rabbit IgG (goat) 2nd antibody DyLight 649, excitation 646 nm); E – immunofluorescence labeling of β II-tubulin (primary antibody: mouse anti-tubulin β II(β 2) (Abcam) at 1/1000, second antibody: Cy 5-conjugated AffiniPure goat anti-mouse IgG (Jackson ImmunoResearch), excitation 633 nm); C, F – intensity plots along white lines drawn through representative sequences of 4 mitochondria (panels A, B, D, E). Dashed red lines indicate the background level of unspecific fluorescence staining measured in control experiments. Red plots are presented in relative units using the same scale for β II-tubulin and α -actinin. Green plots were normalized to the 100% of the maximal intensity of autofluorescence after the background subtraction. Figure reproduced from Gonzalez-Granillo et al., 2011 with permission.

Fig. 4 shows that treatment with 0.8 M KCl changes the organization of tubulins: microtubular network is not any more observed. The intensive immunofluorescence of tubulins is seen in association with cellular structures including mitochondria (Figs. 4B and C). The apparent affinity for ADP of cells incubated with colchicine didn't change significantly in comparison with healthy cardiomyocytes (the app. Km for ADP for ghost cells is about 280 μ M) (Fig. 1) [34]. These experiments indicate the existence of a strong connection between mitochondria and tubulin. However isotypes of $\alpha\beta$ -heterodimer of tubulin interacting with VDAC under physiological and pathological conditions remain uncovered. It is also unknown how this process occurs and how this association influences mitochondrial metabolism.

In intact cells, other cytoskeletal proteins are also shown to form contacts with VDAC in the outer mitochondrial membrane, particularly desmin and plectin [35–39]. Among many known isoforms of plectin, skeletal and cardiac muscles are characterized by high expression of plectins 1, 1b, 1d, and 1f isoforms [39]. Plectin 1d is specifically associated with Z-disks, whereas plectin 1b is shown to be

colocalized with mitochondria. Plectin 1b is inserted into the outer mitochondrial membrane with the exon 1b-encoded N-terminal sequence serving as a mitochondrial targeting and anchoring signal, and may directly interact with voltage-dependent anion channel (VDAC) in mitochondrial membrane. Among other mechanisms influencing the VDAC selective permeability there are posttranslational modifications of tubulins and VDAC [30,40–43], as well as interactions with microtubule-associated protein 2 (MAP2) [22] and cyclic nucleotide phosphodiesterase. Association of the cytoskeletal proteins like β 2-tubulin and plectin with mitochondrial outer membrane evidently may prevent from binding of both pro-apoptotic proteins and those inducing mitochondrial fission or fusion.

3. The influence of VDAC–tubulin interaction on mitochondrial functioning

The VDAC–tubulin interaction participates in the regulation of mitochondrial functioning via the modulation of mitochondrial microcompartmentalization of adenine nucleotides. This indicates, for

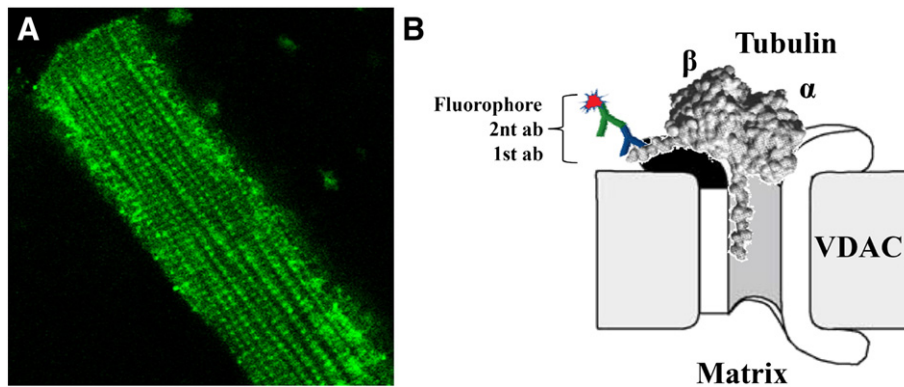


Fig. 3. A – immunofluorescence labeling of β II-tubulin (primary antibody: mouse anti-tubulin β II(β 2) (Abcam) at 1/1000, Goat polyclonal 2nd antibody to Mouse IgG (FITC) (Rockland)), excitation 495 nm). Fluorescent spots are organized in distinct longitudinally oriented parallel lines similar to the mitochondrial arrangement. B – schematic representation of the association of dimeric $\alpha\beta$ -tubulin with VDAC in adult cardiac muscle cells. The β II-tubulin C-terminal tail (CTT) labeled with antibodies (primary, secondary + fluorophore) bind to the VDAC protein binding site situated on the cytosolic surface of porine. One of α -tubulins CTT may penetrate inside the channel decreasing its diameter.

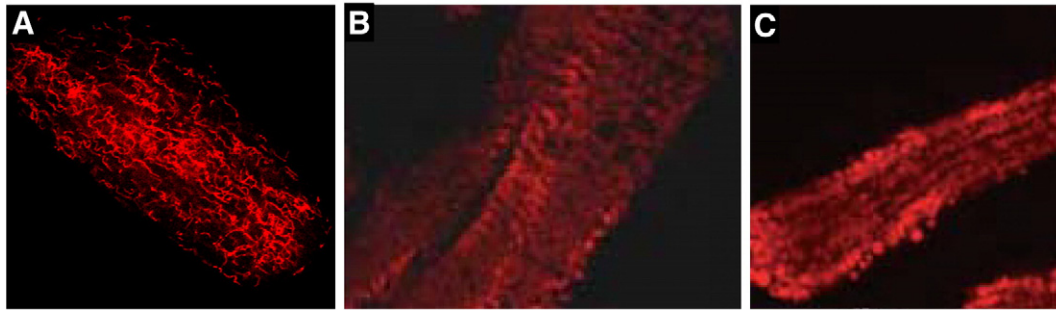


Fig. 4. Mitochondrial and β -tubulin distribution in ghost rat cardiomyocytes. A – immunofluorescence of β -tubulin microtubular network in rat permeabilized cardiomyocytes (monoclonal anti- β -tubulin antibody (Sigma) at 1:200, 2nd antibody rhodamine (TRITC)-conjugated AffiniPure F(ab0)2 fragment Donkey Anti-Mouse IgG, excitation 503 nm). B – immunofluorescence of depolymerized β -tubulin in ghost cardiomyocytes. Tubulin has an ordered striated pattern. C – regular distribution of mitochondria in ghost cardiomyocytes. Mitochondria are labeled by 0.2 μ M MitoTracker Red, excitation 510 nm.

example, the increase of the apparent KmADP after the addition of heterodimeric tubulin to isolated heart mitochondria [27]. Under conditions of the restricted diffusion of adenine nucleotides, the energy of the phosphorylation potential is exported from heart mitochondria by PCr. The last one is produced by mitochondrial creatine kinase (MtCK) from mitochondrial ATP and creatine.

Mitochondrial CK is an octameric protein situated in the contact sites of two mitochondrial boundary membranes, neighboring VDAC at the outer membrane and ANT at the inner membrane (Fig. 5). MtCK binds electrostatically to the negatively charged cardiolipins of the inner membrane gathering and sharing the same cardiolipin patches with ANT [44–47]. Spatial neighboring of MtCK with VDAC is supposed to be realized through the electrostatic binding to the phospholipids of the outer membrane. A spatial association of MtCK with VDAC was shown in digitonin-treated mitochondria and in vitro complex formation of MtCK with VDAC and ANT and directly by surface plasmon resonance [48–52]. The formation of contact sites is a dynamic process highly dependent on the activity of oxidative phosphorylation [53,54]. The isolation and functional reconstitution into vesicles of the VDAC–MtCK–ANT and hexokinase (HK)–VDAC–ANT complexes revealed that the uploaded ATP within vesicles does not get out due to the role of VDAC [52,55]. The dissociation of CK from complex showed the direct interaction of VDAC with ANT (proved by the release of malate from vesicle in the presence of high concentration of Ca²⁺ after the dissociation of CK).

The role of the MOM selective permeability for phosphate compounds can be studied by applying kinetic analysis of MtCK reaction. The respiration of isolated heart mitochondria stimulated by creatine is decreased more than by 50% of its maximal rate in the presence of a powerful system of pyruvate kinase (PK) and phosphoenolpyruvate (PEP) consuming ADP in the milieu [56,57]. This experiment is reproduced in Fig. 6C and means that up to 50% of ADP produced in MtCK reaction leaves the mitochondria via VDAC and 50% is directly transferred via ANT from MtCK to ATP synthase for rephosphorylation (i.e. activation of respiration). The adaptation of this protocol for the study of permeabilized cells allowed us to reveal the role of the VDAC–tubulin interaction for the regulation of oxidative phosphorylation in cardiomyocytes [57,58]. In the permeabilized cardiomyocytes the powerful PK–PEP system is not able to reduce the rate of respiration activated by ATP and creatine (Fig. 6B). This effect can be explained by the inaccessibility of intramitochondrial ADP for the ADP trapping system present in the milieu [12,58]. Additionally this experimental protocol permits to study the MtCK kinetic properties activating MtCK reaction by the stepwise addition of creatine or ATP in the presence of PK and PEP trapping all extramitochondrial ADP (Fig. 6) [58]. The dissociation constants of ATP from binary and ternary MtCK–substrate complexes (K_a and K_{ia} ; Fig. 6) increased from 0.016 mM to 2.04 mM in cardiomyocytes in comparison with isolated mitochondria (Fig. 6). These results indicate the decreased affinity of MtCK for

extramitochondrial ATP due to the decreased VDAC permeability in permeabilized cardiomyocytes. In contrast, the dissociation constants for PCr from MtCK–substrate complex were quasi similar in permeabilized cardiomyocytes and isolated heart mitochondria, about 0.89 mM, and those of creatine decreased slightly from 5 mM for isolated heart mitochondria to 2.17 mM for cardiomyocytes (Fig. 6). This indicates free diffusion of creatine and PCr throughout MOM for both models [12,58]. Because of this rather selective control of VDAC permeability and functional coupling between MtCK and ANT, all ATP produced in oxidative phosphorylation is practically completely used for PCr production and ADP is rapidly channeled back through ANT to the mitochondrial matrix. Direct measurements of energy fluxes from mitochondria into cytoplasm (surrounding medium in experiments with permeabilized cardiac cells), performed by Timohinna et al., in 2009, have shown that the PCr/O₂ ratio is close to the theoretical maximal P/O₂ ratio under conditions similar to those in vivo (P/O₂ ratio ~6) [12]. These results are in agreement with Dzeja et al., studies of the intracellular energy transfer in rat heart. Authors have shown that the net CK-catalyzed phosphoryl transfer is equivalent to about 80–88% of the total ATP turnover rate as measured by 18O-phosphoryl labeling [59,60]. Once again, this experiment shows that the permeability of VDAC in MOM in permeabilized cardiomyocytes is strongly decreased for adenine nucleotides and that this MOM property can be influenced from inside due to the VDAC interaction with MtCK as well as from outside of mitochondria due to the VDAC interactions with cytosolic proteins. Thus, the results of functional studies are reminiscent for the existence of the interaction of VDAC with extra- and intra-mitochondrial proteins capable to selectively regulate VDAC permeability for ATP, ADP, PCr and creatine.

4. Energy fluxes between different cellular compartments

Studying intracellular phosphotransfer using ¹⁸O labeling of the phosphoryl groups, in 1996 Dzeja et al. have shown that about 80–88% of high-energy phosphoryl transfer in healthy adult cardiac muscle cells is provided by compartmentalized CK reactions and about 7–20% of the total ATP rate is supplied by compartmentalized adenylate kinase (AK) reactions [61]. The net rates of AK catalyzed phosphoryl transfer (the appearance of each newly synthesized molecule of ADP and upon its phosphorylation, ATP) increased from the equivalent of 2–3% of the total ATP flux in the resting muscle to 23% of the ADP produced in ATPase reaction during contraction [62–64]. Compartmentalized AK reactions are realized by mitochondrial AK (isoforms 2 and 3) and cytosolic AK (isoform 1). Cytosolic AK catalyzes the transfers of high energy phosphoryls of ADP (Fig. 7). Produced ATP is subsequently hydrolyzed by ATPase to realize work while the AMP is returned back to mitochondria as the metabolic signal. Measuring the ¹⁸O-phosphoryl oxygen exchanges, Dzeja et al.

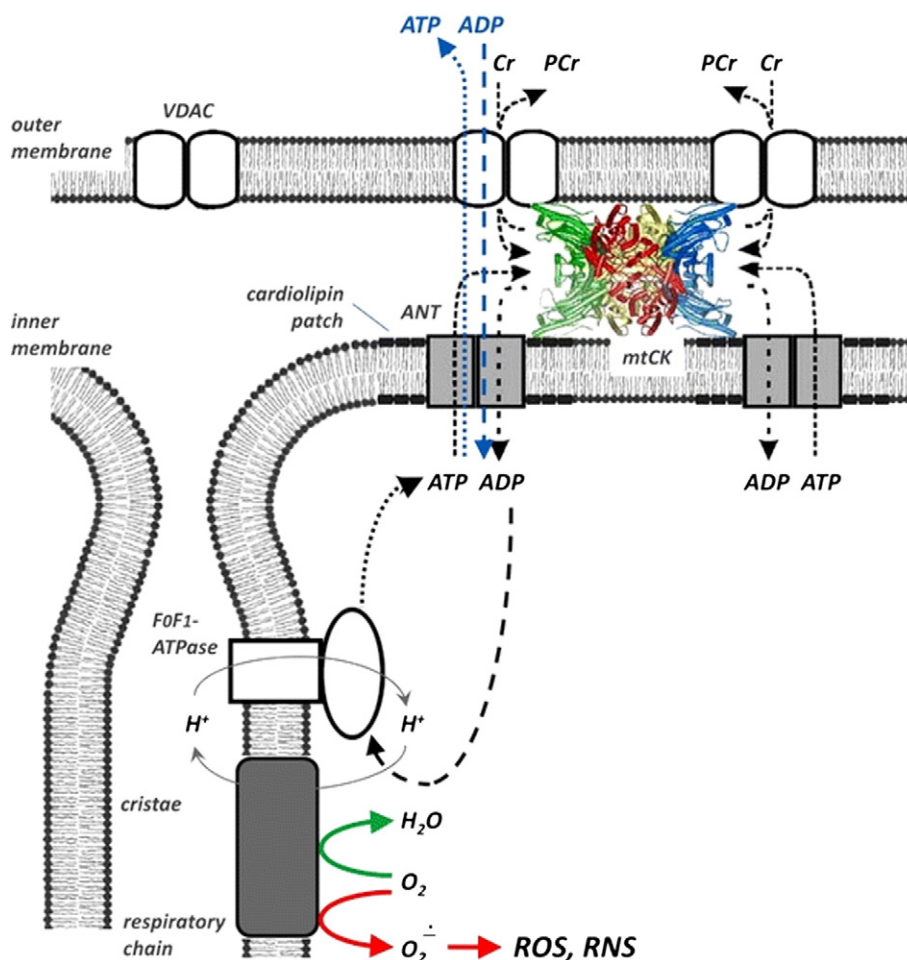


Fig. 5. Mitochondrial MtCK functions for high-energy metabolite channeling in mitochondria. In cells with oxidative metabolism, respiration (green arrow), ATP synthesis and ATP export through the inner mitochondrial membrane via adenine nucleotide transporter (ANT) are tightly coupled to trans-phosphorylation of ATP to PCr by MtCK and export of PCr into the cytosol by the outer membrane voltage-dependent anion channel (VDAC) as indicated by black arrows. The structural basis of these MtCK microcompartments are proteolipid complexes containing either VDAC, octameric MtCK and ANT in the peripheral intermembrane space (as shown) or octameric MtCK and ANT in the cristae (not shown). These proteolipid complexes are maintained by MtCK interactions with anionic phospholipids and VDAC in the outer membrane, and with cardiolipin and thus indirectly with cardiolipin-associated ANT in the inner membrane (see cardiolipin patches). Reproduced with permission from Wallimann et al., 2011.

showed in 1998 that the rate of AMP phosphorylation increases with a raised frequency of muscle contraction while tissue concentrations of ATP, ADP and AMP exhibited virtual constancy [64]. According to the mechanism of ligand conduction discovered by P. Mitchell 1974 and applied by Dzeja et al. 1998 for the phosphotransfer circuits, the input of one molecule of AMP in the myofibrillar end of the system of compartmentalized AK reactions will result in the output of one AMP molecule at the mitochondrial end triggering the AK-catalyzed phosphorylation of AMP. Two molecules of ADP produced in AK2 reaction are rephosphorylated within the matrix. Resynthesized ATP follows the way of MtCK reaction, amplifying PCr production. As a result, the AMP entering mitochondria amplifies both the high-energy transfer and the intramitochondrial ADP recycling through functional coupling of oxidative phosphorylation with MtCK. Thus the system of compartmentalized AK reactions translates small changes in the ATP/ADP ratio through the minimal AMP signaling into relatively large changes of the high-energy phosphoryl supply [65,66]. The VDAC selective permeability for creatine and phosphocreatine plays a crucial role in the distribution of the energy fluxes and metabolic signals.

All data described in the review is summarized by a general Scheme in Fig. 7. This shows the Mitochondrial Interactosome, a supercomplex structurally constituted of ATP synthase which interacts on its one side with the electron transport chain and on the other with the peripheral energy consuming sites through ANT,

MtCK and VDAC with cytoskeleton, particularly tubulins [10–12]. The role of this functional and structural supercomplex is to increase the efficiency of the oxidative ATP synthesis and peripheral ATP hydrolysis through the generation of two circuits allowing cells to avoid the waste of energy and to return the metabolic signal fast enough to fulfill energy requirement. One of them is the intramitochondrial circuit of ATP/ADP between ATP synthase and MtCK due to their functional coupling via ANT. MtCK catalyzes the phosphotransfer from mitochondrial ATP to creatine, returning back into the matrix ADP for rephosphorylation. The second circuit is that of creatine/phosphocreatine between different isotypes of creatine kinase: mitochondrial CK producing PCr from mitochondrial ATP and cytosolic CK locally regenerating ATP from ADP and PCr. These structures represent a new type of supramolecular organization that operates far from equilibrium conditions. They were defined by De la Fuente as cellular metabolic dissipative structures, representing functional enzymatic associations that form a catalytic entity as a whole and carry out their activities in a relatively independent manner [67,68].

5. Metabolic control analysis within Mitochondrial Interactosome

The selective VDAC permeability for ATP/ADP and Cr/PCr has a significant impact on the control of the respiration rate and energy fluxes displayed by different components of the Mitochondrial

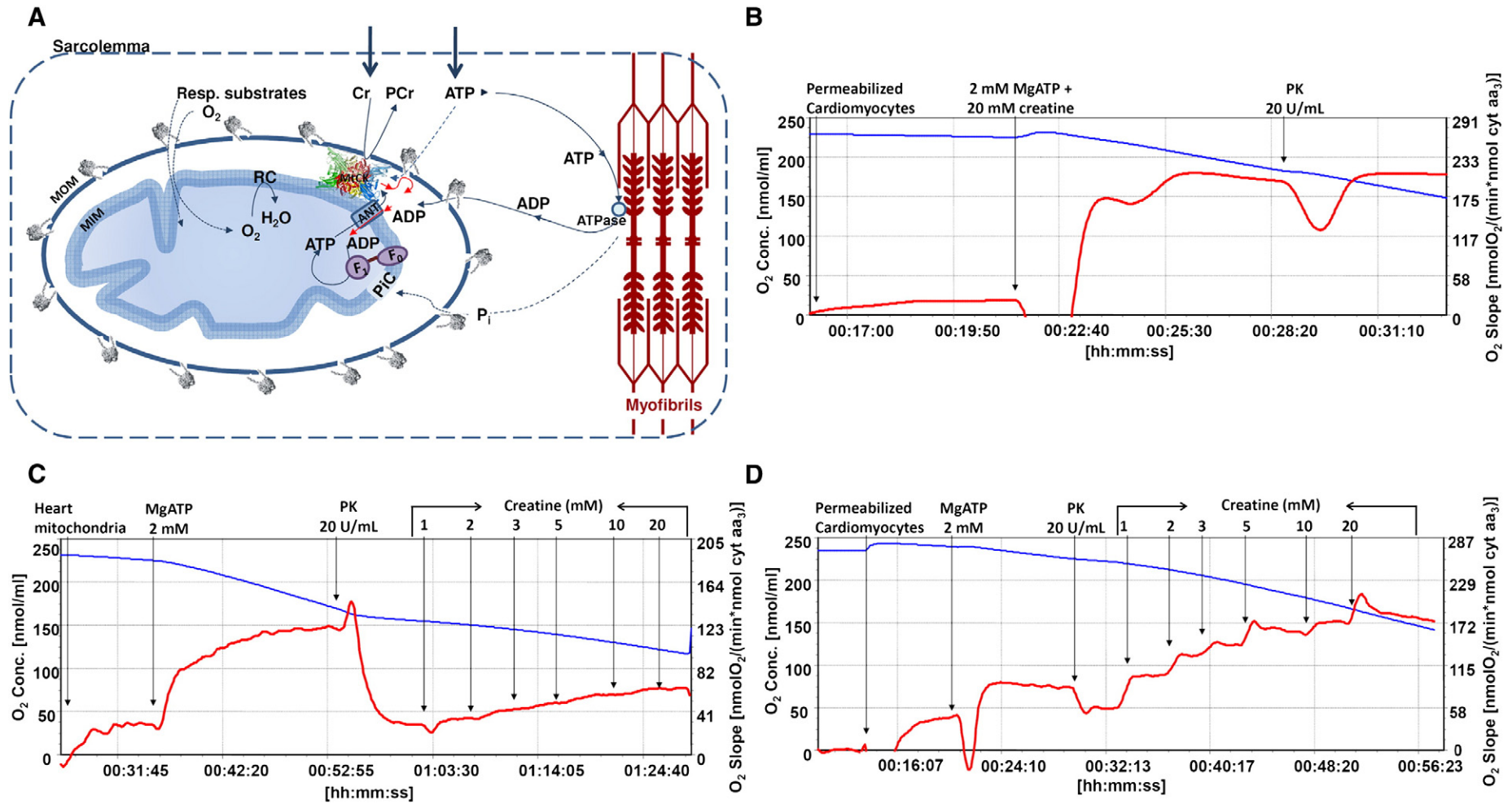


Fig. 6. Regulation of mitochondrial respiration by creatine. The influence of mitochondrial outer membrane permeability on the functional coupling of mitochondrial creatine kinase (MtCK) with adenine nucleotide translocase (ANT). A, C and D show the protocol and experimental oxygraph recording of the respiration rate regulated by ADP produced by MtCK in isolated mitochondria (C) and permeabilized cardiomyocytes (D). First, the basal rate of respiration (V_0) of permeabilized cardiomyocytes (25 $\mu\text{g}/\text{mL}$ saponin, 10 min, 25 $^\circ\text{C}$) was recorded in the presence of respiratory substrates for complex I (5 mM glutamate and 2 mM malate). After that, respiration was activated by the addition of 2 mM MgATP which is hydrolyzed by myofibrillar ATPases releasing endogenous MgADP, inorganic phosphate (P_i) and proton. On the oxygraph trace this step is seen as an increase in oxygen consumption stimulated by endogenous MgADP. Next, extramitochondrial MgADP was removed by the addition of phosphoenolpyruvate (PEP, 5 mM) and pyruvate kinase (PK, 20 IU/mL). The PEP dephosphorylation catalyzed by PK uses ADP to regenerate extramitochondrial pool of ATP. This step is seen on oxygraph trace as an inhibition of respiration. Finally, stepwise addition of creatine in the presence of MgATP activates MtCK and in thus MgADP and PCr production from MgATP and creatine. Figure (B) shows the full compartmentalization of intramitochondrial ADP produced by MtCK in the intermembrane space of mitochondria in permeabilized cardiomyocytes in the presence of activated MtCK by the addition of 2 mM ATP and 20 mM creatine. Addition of PK in the presence of PEP (5 mM added into medium before) did not change significantly the respiration rate. In mitochondria in situ when respiration is controlled by the MtCK reaction, the powerful PEP–PK system is not able to inhibit respiration. The permeability of VDAC for ADP seems to be strongly decreased.

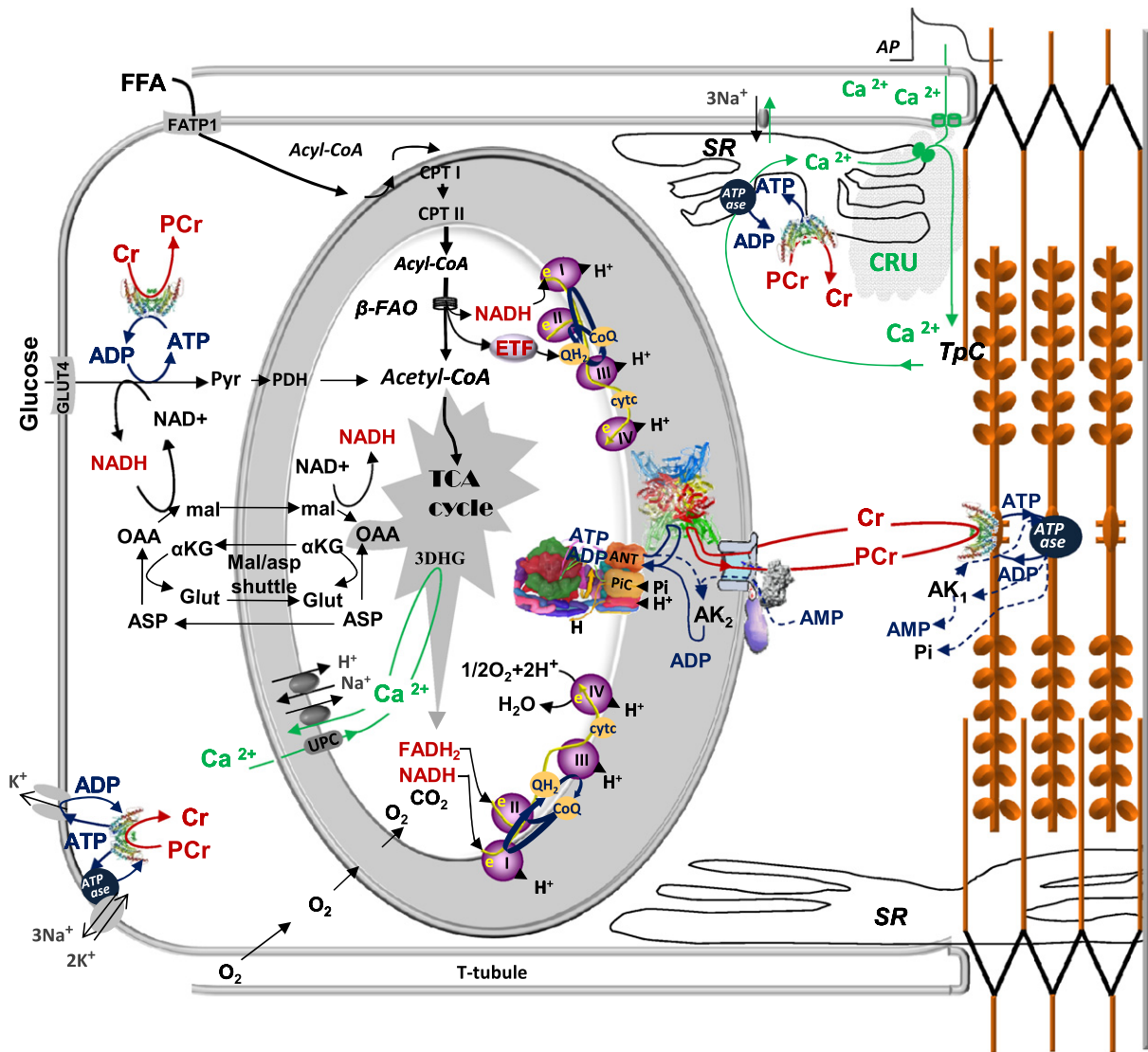


Fig. 7. Functional scheme of the Intracellular Energetic Units of adult cardiac muscle cell. Free fatty acids (FFA) are esterified to acyl-CoA which entering further the β -fatty acids oxidation (β -FAO) pathway results in acetyl-CoA production. CPT I and CPT II – carnitine palmitoyltransferases. Electron-transferring flavoprotein (ETF)-ubiquinone oxidoreductase delivers electrons from β -FAO directly to complex III of the respiratory chain (RC). NADH produced by β -FAO is oxidized in the complex I of the RC passing along two electrons and two protons which contribute to the polarization of mitochondrial inner membrane (MIM). Glucose (GLU) is taken up by glucose transporter-4 (GLUT-4) and oxidized via Embden–Meyerhof pathway. Pyruvate produced from glucose oxidation is transformed by the pyruvate dehydrogenase complex (PDH) into acetyl-CoA. The NADH redox potential resulted from glycolysis enters mitochondrial matrix via malate-aspartate shuttle. Acetyl-CoA is oxidized to CO_2 in the tricarboxylic acids (TCA) cycle generating NADH and FADH_2 which are further oxidized in the RC (complexes I, II) with final ATP synthesis. The key system in energy transfer from mitochondria to cytoplasm is Mitochondrial Intersacosome (MI). MI is a supercomplex, formed by ATP synthase, adenine nucleotides translocase (ANT), phosphate carriers (PIC), mitochondrial creatine kinase (MtCK), voltage-dependent anion channel (VDAC) with bound cytoskeleton proteins (specifically β II-tubulin). MI is responsible for the narrow coupling of ATP/ADP intramitochondria turnover with phosphorylation of creatine (Cr) into phosphocreatine (PCr). PCr is then used to regenerate ATP locally by CK with ATPases (actomyosin ATPase, sarcoplasmic reticulum SERCA and ion pumps ATPases). The rephosphorylation of ADP in MMCK reaction increases the Cr/PCr ratio which is transferred toward MtCK via CK/PCr shuttle. A small part of ADP issued from ATP hydrolysis creates gradient of concentration transmitted toward the matrix and is transphosphorylated by adenylate kinase (AK) with local regeneration of ATP and AMP production. The AMP transmitted to mitochondria via the system of compartmentalized AK reactions is re-phosphorylated by mitochondrial AK using ATP. Two molecules of ADP produced in AK reaction are transferred into the matrix for rephosphorylation. The high-energy phosphoryls of newly synthesized ATP are exported from mitochondria as PCr due to the functional coupling of ATP synthase with MtCK via ANT. The shaded area in the upper right corner shows the Calcium Release Unit. Figure adapted from Saks et al., 2011 with permission.

Interactosome. As recent as 2011, Tepp et al., published their results of the study on flux control coefficients (FCC) of different components of MI in isolated rat heart mitochondria and permeabilized cardiomyocytes under conditions of activated MtCK [69]. The MtCK was activated by addition of creatine and MgATP in the presence of a system trapping extramitochondrial ADP consisting of PK and PEP [57]. The contribution of ANT and MtCK in the control of respiration rate was studied by gradually inhibiting every component with carboxyatractylate and 1-fluoro-2,4-dinitrobenzene, respectively. Authors have shown that the FCC increased in permeabilized cardiomyocytes in comparison with isolated heart mitochondria from 0.38 ± 0.07 to

0.92 ± 0.05 for ANT and from 0.80 ± 0.04 to 0.95 ± 0.02 for MtCK [70]. The sum of the flux control coefficients of respiratory chain complexes, ATP synthase, MtCK, ANT, and metabolite carriers (phosphate carrier inclusively) in isolated heart mitochondria is close to 1 and in permeabilized cardiomyocytes is close to 3.8 [69–71]. The significant increase of FCC that MI components exert on the respiratory rate in cardiomyocytes is consistent with the enhanced functional coupling of MtCK–ANT with intramitochondrial ADP recycling under conditions of adenine metabolite compartmentalization created by the mitochondrial interaction with the intracellular environment.

6. The VDAC selective permeability in physiological and pathological states

Going back to the Frank-Starling's law of the heart, with which we started the in introduction part, we can now explain the mechanism allowing the linear dependence between the increase of left ventricular end-diastolic volume and the increase of respiration rates in the absence of measurable changes in the intracellular ATP and PCr content. According to the Honda's experimental data and Aliev-Saks-Vendelin's mathematical model there is a significant change in ADP during the contraction cycle in the myofibrillar space under conditions of the metabolic stability [72–74]. Minimal ADP levels (close to the equilibrium values of ADP concentration, i.e. 50 μM) are characteristic for the diastolic phase of the contraction cycle, and the peak values of ADP (within the physiological ranges, i.e. up to 300–500 μM) increase with elevation of workload and, subsequently, of VO_2 . The kinetic analysis of the regulation of cardiomyocytes respiration shows that due to the restricted ADP diffusion at the level of MOM (app. KmADP \sim 400 μM), the respiration rates become linearly dependant on ADP concentrations (in the physiological ranges, 50–500 μM), in effect dependent on heart workloads in accordance with the Frank-Starling law. This linear dependence under physiological conditions can be amplified by creatine in the presence of activated MtCK due to the local intramitochondrial recycling of ADP in the reaction of the PCr production catalyzed by MtCK (the app. KmADP decreases in the presence of creatine up to 20–40 μM) [10,11,58]. This means that the small (signaling) amounts of cytosolic ADP entering mitochondria and re-phosphorylated by ATP synthase into ATP, follow the pathway of MtCK reaction which via the repetitive recycling of intramitochondrial ADP (with PCr production) amplifies the cytosolic ADP signal. This effect was confirmed quantitatively by the means of Metabolic Control Analysis. Studying the FCC of ANT under conditions of ADP-stimulated respiration and in the presence of activated MtCK (as described above) Tepp et al., (2011) have shown that the FCC of ANT increased from 0.2 to 0.92 when respiration was stimulated by endogenous ADP recycled in the activated MtCK reaction [69].

Previously we have shown that mitochondrial oxidative phosphorylation of cancerous cardiac cells (NB HL-1) displays a very high apparent affinity for exogenous ADP. The apparent KmADP is comparable with that of isolated mitochondria, about 8–12 μM [75]. What is most interesting regarding the topic of our review-article discussed here is the simultaneous absence of MtCK and β 2-tubulin in NB HL-1 cells. The absence of these two key proteins of the Mitochondrial Interactosome coincides with a difference in mitochondrial distribution in NB HL-1 cells, characterized by reticular, rapidly fusing-fissioning mitochondria [8] and high glycolytic enzyme activities [76] distinct from healthy cardiomyocytes. According to Patra et al., the absence of MtCK is a characteristic of several cancer cells [77,78]. At the same time, the absence of β 2-tubulin in many types of cancer cells was previously found using Western blot analysis. The mitochondrial respiration of NB HL-1 cells is highly sensitive for ADP and glucose due to the interaction of VDAC with HK2 [79]. For this type of cells, for which the oxidative phosphorylation is directly regulated by extramitochondrial ADP, the effect described by Maldonado et al. (2010) becomes relevant. Maldonado showed the loss of membrane potential in cancerous hepatocytes induced by depolymerization of microtubular network [80]. According to authors, small polymers of tubulin can interact with VDAC, decreasing its permeability for ADP/ATP and thus mitochondrial membrane potential. Certainly, restricted ADP/ATP diffusion at the level of MOM can be harmful for cells with mitochondrial respiration stimulated notably by cytosolic ADP and missing (or insufficiently represented) alternative phosphotransfer networks (such as CK, AK, guanine nucleotide phosphotransfer). In contrast, in healthy adult cardiomyocytes in which up to 88% of phosphorylation potential energy is transferred

via the CK/PCr network, the restriction of ADP/ATP diffusion is beneficial. This is why, colchicine doesn't have the same effect on cardiomyocytes as on cancerous hepatocytes. The influence of VDAC permeability on mitochondrial metabolism has to be evaluated in the whole context of cellular metabolism and mechanisms of regulation.

Despite these evidences some questions still persist about the structure and functioning of the Mitochondrial Interactosome of non-cancerous glycolytic cells. Do they keep their non-cancerous profile because of the restricted VDAC permeability for adenine nucleotides induced by the interaction of VDAC with some proteins (like β 2-tubulin and MtCK in myocytes)? Does this interaction protect VDAC from the HK binding and control from the energy outflow as it is characteristic for cancer cells? The answer to these questions is fundamental and could stimulate research to resolving significant cancer bioenergetics problems.

Acknowledgements

This work was supported by INSERM, France, by Agence Nationale de la Recherche, project ANR N RA0000C407, France, by grant no. 7823 from the Estonian Science Foundation, SF0180114Bs08 from Estonia Ministry of Education and Science, by National Council of Science and Technology of Mexico (CONACYT) and by a research grant from the Austrian Science Fund (FWF): [P 22080-B20].

References

- [1] O. Frank, Zur Dynamik des Herzmuskels, *Z. Biol.* 32 (1885) 370–447.
- [2] E.H. Starling, M.B. Visscher, The regulation of the energy output of the heart, *J. Physiol.* 62 (1927) 243–261.
- [3] V. Saks, P. Dzeja, U. Schlattner, M. Vendelin, A. Terzic, T. Wallimann, Cardiac system bioenergetics: metabolic basis of the Frank-Starling law, *J. Physiol.* 571 (2006) 253–273.
- [4] R.S. Balaban, H.L. Kantor, L.A. Katz, R.W. Briggs, Relation between work and phosphate metabolite in the in vivo paced mammalian heart, *Science* 232 (1986) 1121–1123.
- [5] L.A. Katz, J.A. Swain, M.A. Portman, R.S. Balaban, Relation between phosphate metabolites and oxygen consumption of heart in vivo, *Am. J. Physiol.* 256 (1989) H265–H274.
- [6] S. Gudbjarnason, P. Mathes, K.G. Ravens, Functional compartmentation of ATP and creatine phosphate in heart muscle, *J. Mol. Cell. Cardiol.* 1 (1970) 325–339.
- [7] M. Vendelin, N. Beraud, K. Guerrero, T. Andrienko, A.V. Kuznetsov, J. Olivares, L. Kay, V.A. Saks, Mitochondrial regular arrangement in muscle cells: a "crystal-like" pattern, *Am. J. Physiol. Cell Physiol.* 288 (2005) C757–C767.
- [8] N. Beraud, S. Pelloux, Y. Usson, A.V. Kuznetsov, X. Ronot, Y. Tourneur, V. Saks, Mitochondrial dynamics in heart cells: very low amplitude high frequency fluctuations in adult cardiomyocytes and flow motion in non beating HL-1 cells, *J. Bioenerg. Biomembr.* 41 (2009) 195–214.
- [9] V. Saks, C. Monge, T. Anmann, P. Dzeja, Integrated and organized cellular energetics: theories of cell energetics, compartmentation and metabolic channeling, in: V. Saks (Ed.), *Molecular System Bioenergetics. Energy for Life*, Wiley-VCH, Weinheim, GmbH, Germany, 2007, pp. 59–110.
- [10] V. Saks, R. Guzun, N. Timohhina, K. Tepp, M. Varikmaa, C. Monge, N. Beraud, T. Kaambre, A. Kuznetsov, L. Kadaja, M. Eimre, E. Seppet, Structure-function relationships in feedback regulation of energy fluxes in vivo in health and disease: Mitochondrial Interactosome, *Biochim. Biophys. Acta* 1797 (2010) 678–697.
- [11] V. Saks, A.V. Kuznetsov, M. Gonzalez-Granillo, K. Tepp, N. Timohhina, M. Karu-Varikmaa, T. Kaambre, P.D. Santos, F. Boucher, R. Guzun, Intracellular Energetic Units regulate metabolism in cardiac cells, *J. Mol. Cell. Cardiol.* doi:10.1016/j.yjmcc.2011.07.015 [2011 Jul 26, Electronic publication ahead of print].
- [12] N. Timohhina, R. Guzun, K. Tepp, C. Monge, M. Varikmaa, H. Vija, P. Sikk, T. Kaambre, D. Sackett, V. Saks, Direct measurement of energy fluxes from mitochondria into cytoplasm in permeabilized cardiac cells in situ: some evidence for Mitochondrial Interactosome, *J. Bioenerg. Biomembr.* 41 (2009) 259–275.
- [13] Y.H. Ko, M. Delannoy, J. Hüllihen, W. Chiu, P.L. Pedersen, Mitochondrial ATP synthasome, Cristae-enriched membranes and a multiwell detergent screening assay yield dispersed single complexes containing the ATP synthase and carriers for Pi and ADP/ATP, *J. Biol. Chem.* 278 (2003) 12305–12309.
- [14] V. Saks, Y. Belikova, E. Vasilyeva, A. Kuznetsov, E. Fontaine, C. Keriel, X. Leverve, Correlation between degree of rupture of outer mitochondrial membrane and changes of kinetics of regulation of respiration by ADP in permeabilized heart and liver cells, *Biochem. Biophys. Res. Commun.* 208 (1995) 919–926.
- [15] P. Konieczny, P. Fuchs, S. Reipert, W.S. Kunz, A. Zeold, I. Fischer, D. Paulin, R. Schroder, G. Wiche, Myofiber integrity depends on desmin network targeting to Z-disks and costameres via distinct plectin isoforms, *J. Cell Biol.* 181 (2008) 667–681.
- [16] P. Konieczny, G. Wiche, Muscular integrity—a matter of interlinking distinct structures via plectin, *Adv. Exp. Med. Biol.* 642 (2008) 165–175.

- [17] G.A. Reznicek, C. Abrahamsberg, P. Fuchs, D. Spazierer, G. Wiche, Plectin 5'-transcript diversity: short alternative sequences determine stability of gene products, initiation of translation and subcellular localization of isoforms, *Hum. Mol. Genet.* 12 (2003) 3181–3194.
- [18] G.A. Reznicek, P. Konieczny, B. Nikolic, S. Reipert, D. Schneller, C. Abrahamsberg, K.E. Davies, S.J. Winder, G. Wiche, Plectin 1f scaffolding at the sarcolemma of dystrophic (mdx) muscle fibers through multiple interactions with beta-dystroglycan, *J. Cell Biol.* 176 (2007) 965–977.
- [19] L. Winter, C. Abrahamsberg, G. Wiche, Plectin isoform 1b mediates mitochondrion-intermediate filament network linkage and controls organelle shape, *J. Cell Biol.* 181 (2008) 903–911.
- [20] M. Carre, N. Andre, G. Carles, H. Borghi, L. Brichese, C. Briand, D. Braguer, Tubulin is an inherent component of mitochondrial membranes that interacts with the voltage-dependent anion channel, *J. Biol. Chem.* 277 (2002) 33664–33669.
- [21] T. Saetersdal, G. Greve, H. Dalen, Associations between beta-tubulin and mitochondria in adult isolated heart myocytes as shown by immunofluorescence and immunoelectron microscopy, *Histochemistry* 95 (1990) 1–10.
- [22] M. Linden, G. Karlsson, Identification of porin as a binding site for MAP2, *Biochem. Biophys. Res. Commun.* 218 (1996) 833–836.
- [23] T.K. Rostovtseva, S.M. Bezrukov, VDAC regulation: role of cytosolic proteins and mitochondrial lipids, *J. Bioenerg. Biomembr.* 40 (2008) 163–170.
- [24] T.K. Rostovtseva, K.L. Sheldon, E. Hassanzadeh, C. Monge, V. Saks, S.M. Bezrukov, D.L. Sackett, Tubulin binding blocks mitochondrial voltage-dependent anion channel and regulates respiration, *Proc. Natl. Acad. Sci. U. S. A.* 105 (2008) 18746–18751.
- [25] T. Rostovtseva, Control of mitochondrial outer membrane permeability: VDAC regulation by dimeric tubulin and cytosolic proteins, in: O.L. Svensson (Ed.), *Mitochondria: Structure, Functions and Dysfunctions*, Nova Biomedical books, New York, 2010, pp. 607–634.
- [26] T.K. Rostovtseva, S.M. Bezrukov, VDAC inhibition by tubulin and its physiological implications, *Biochim. Biophys. Acta*, doi:10.1016/j.bbame.2011.11.004 [2011 Nov 9, Electronic publication ahead of print].
- [27] C. Monge, N. Beraud, A.V. Kuznetsov, T. Rostovtseva, D. Sackett, U. Schlattner, M. Vendelin, V.A. Saks, Regulation of respiration in brain mitochondria and synaptosomes: restrictions of ADP diffusion in situ, roles of tubulin, and mitochondrial creatine kinase, *Mol. Cell. Biochem.* 318 (2008) 147–165.
- [28] R. Guzun, M. Karu-Varikmaa, M. Gonzalez-Granillo, A.V. Kuznetsov, L. Michel, C. Cotte-Rousselle, M. Saaremaa, T. Kaambre, M. Metsis, M. Grimm, C. Auffray, V. Saks, Mitochondria-cytoskeleton interaction: distribution of beta-tubulins in cardiomyocytes and HL-1 cells, *Biochim. Biophys. Acta* 1807 (2011) 458–469.
- [29] M. Gonzalez-Granillo, A. Grichine, R. Guzun, Y. Usson, K. Tepp, V. Chekulayev, I. Shevchuk, M. Karu-Varikmaa, A.V. Kuznetsov, M. Grimm, V. Saks, T. Kaambre, Studies of the role of tubulin beta II isotype in regulation of mitochondrial respiration in intracellular energetic units in cardiac cells, *J. Mol. Cell. Cardiol.* doi:10.1016/j.yjmcc.2011.07.027 [2011 Aug 5, Electronic publication ahead of print].
- [30] D. Sackett, Evolution and coevolution of tubulin's carboxy-terminal tails and mitochondria, in: O.L. Svensson (Ed.), *Mitochondria: Structure, Function and Dysfunction*, Nova Biomedical Books, New York, 2010, pp. 789–810.
- [31] J. Duan, M.A. Gorovsky, Both carboxy-terminal tails of alpha- and beta-tubulin are essential, but either one will suffice, *Curr. Biol.* 12 (2002) 313–316.
- [32] M. Colombini, VDAC: the channel at the interface between mitochondria and the cytosol, *Mol. Cell. Biochem.* 256–257 (2004) 107–115.
- [33] M. Colombini, The published 3D structure of the VDAC channel: native or not? *Trends Biochem. Sci.* 34 (2009) 382–389.
- [34] K. Guerrero, C. Monge, A. Bruckner, U. Puurand, L. Kadaja, T. Kaambre, E. Seppet, V. Saks, Study of possible interactions of tubulin, microtubular network, and STOP protein with mitochondria in muscle cells, *Mol. Cell. Biochem.* 337 (2010) 239–249.
- [35] Y. Capetanaki, R.J. Bloch, A. Kouloumenta, M. Mavroidis, S. Psarras, Muscle intermediate filaments and their links to membranes and membranous organelles, *Exp. Cell Res.* 313 (2007) 2063–2076.
- [36] Y. Capetanaki, Desmin cytoskeleton: a potential regulator of muscle mitochondrial behaviour and function, *Trends Cardiovasc. Med.* 12 (2002) 339–348.
- [37] M. Linden, Z. Li, D. Paulin, T. Gotow, J.F. Leterrier, Effects of desmin gene knockout on mice heart mitochondria, *J. Bioenerg. Biomembr.* 33 (2001) 333–341.
- [38] S. Reipert, F. Steinbock, I. Fischer, R.E. Bittner, A. Zeold, G. Wiche, Association of mitochondria with plectin and desmin intermediate filaments in striated muscle, *Exp. Cell Res.* 252 (1999) 479–491.
- [39] R. Schroder, W.S. Kunz, F. Rouan, E. Pfendner, K. Tolksdorf, K. Kappes-Horn, M. Altensmidt-Mehring, R. Knoblich, P.F. van der Ven, J. Reimann, D.O. Furst, I. Blumcke, S. Vielhaber, D. Zillikens, S. Eming, T. Klockgether, J. Uitto, G. Wiche, A. Rolfs, Disorganization of the desmin cytoskeleton and mitochondrial dysfunction in plectin-related epidermolysis bullosa simplex with muscular dystrophy, *J. Neuropathol. Exp. Neurol.* 61 (2002) 520–530.
- [40] K.L. Sheldon, E.N. Maldonado, J.J. Lemasters, T.K. Rostovtseva, S.M. Bezrukov, Phosphorylation of voltage-dependent anion channel by serine/threonine kinases governs its interaction with tubulin, *PLoS One* 6 (2011) e25539.
- [41] M. Bifulco, C. Iaezza, S. Stingo, J. Wolff, 2',3'-Cyclic nucleotide 3'-phosphodiesterase: a membrane-bound, microtubule-associated protein and membrane anchor for tubulin, *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002) 1807–1812.
- [42] J.G. Pastorino, J.B. Hoek, Regulation of hexokinase binding to VDAC, *J. Bioenerg. Biomembr.* 40 (2008) 171–182.
- [43] J.G. Pastorino, J.B. Hoek, N. Shulga, Activation of glycogen synthase kinase 3beta disrupts the binding of hexokinase II to mitochondria by phosphorylating voltage-dependent anion channel and potentiates chemotherapy-induced cytotoxicity, *Cancer Res.* 65 (2005) 10545–10554.
- [44] T. Wallimann, M. Tokarska-Schlattner, U. Schlattner, The creatine kinase system and pleiotropic effects of creatine, *Amino Acids* 40 (2011) 1271–1296.
- [45] U. Schlattner, T. Wallimann, Metabolic channeling: creatine kinase microcompartments, in: W.J. Lennarz, M.D. Lane (Eds.), *Encyclopedia of Biological Chemistry*, Academic Press, New York, USA, 2004, pp. 646–651.
- [46] U. Schlattner, M. Tokarska-Schlattner, T. Wallimann, in: C. Vial (Ed.), *Molecular Structure and Function of Mitochondrial Creatine Kinases*, Creatine kinase Nova Science Publishers, New York, 2006, pp. 123–170.
- [47] U. Schlattner, M. Forstner, M. Eder, O. Stachowiak, K. Fritz-Wolf, T. Wallimann, Functional aspects of the X-ray structure of mitochondrial creatine kinase: a molecular physiology approach, *Mol. Cell. Biochem.* 184 (1998) 125–140.
- [48] M. Kottke, V. Adams, T. Wallimann, V.K. Nalam, D. Brdiczka, Location and regulation of octameric mitochondrial creatine kinase in the contact sites, *Biochim. Biophys. Acta* 1061 (1991) 215–225.
- [49] D. Brdiczka, P. Kaldis, T. Wallimann, In vitro complex formation between the octamer of mitochondrial creatine kinase and porin, *J. Biol. Chem.* 269 (1994) 27640–27644.
- [50] D. Brdiczka, Mitochondrial VDAC and its complexes, in: V. Saks (Ed.), *Molecular System Bioenergetics. Energy for Life*, Wiley-VCH, Weinheim, GmbH, Germany, 2007, pp. 165–194.
- [51] U. Schlattner, M. Dolder, T. Wallimann, M. Tokarska-Schlattner, Mitochondrial creatine kinase and mitochondrial outer membrane porin show a direct interaction that is modulated by calcium, *J. Biol. Chem.* 276 (2001) 48027–48030.
- [52] G. Beutner, A. Ruck, B. Riede, W. Welte, D. Brdiczka, Complexes between kinases, mitochondrial porin and adenylate translocator in rat brain resemble the permeability transition pore, *FEBS Lett.* 396 (1996) 189–195.
- [53] G. Knoll, D. Brdiczka, Changes in freeze-fractured mitochondrial membranes correlated to their energetic state. Dynamic interactions of the boundary membranes, *Biochim. Biophys. Acta* 733 (1983) 102–110.
- [54] K. Bucheler, V. Adams, D. Brdiczka, Localization of the ATP/ADP translocator in the inner membrane and regulation of contact sites between mitochondrial envelope membranes by ADP. A study on freeze-fractured isolated liver mitochondria, *Biochim. Biophys. Acta* 1056 (1991) 233–242.
- [55] M.Y. Vyssokikh, D. Brdiczka, The function of complexes between the outer mitochondrial membrane pore (VDAC) and the adenine nucleotide translocase in regulation of energy metabolism and apoptosis, *Acta Biochim. Pol.* 50 (2003) 389–404.
- [56] W.E. Jacobus, V.A. Saks, Creatine kinase of heart mitochondria: changes in its kinetic properties induced by coupling to oxidative phosphorylation, *Arch. Biochem. Biophys.* 219 (1982) 167–178.
- [57] F. Gellerich, V.A. Saks, Control of heart mitochondrial oxygen consumption by creatine kinase: the importance of enzyme localization, *Biochem. Biophys. Res. Commun.* 105 (1982) 1473–1481.
- [58] R. Guzun, N. Timohhina, K. Tepp, C. Monge, T. Kaambre, P. Sikk, A.V. Kuznetsov, C. Pison, V. Saks, Regulation of respiration controlled by mitochondrial creatine kinase in permeabilized cardiac cells in situ. Importance of system level properties, *Biochim. Biophys. Acta* 1787 (2009) 1089–1105.
- [59] P.P. Dzeja, K. Hoyer, R. Tian, S. Zhang, E. Nemutlu, M. Spindler, J.S. Ingwall, Rearrangement of energetic and substrate utilization networks compensate for chronic myocardial creatine kinase deficiency, *J. Physiol.* 589 (2011) 5193–5211.
- [60] P.P. Dzeja, A. Terzic, Phosphotransfer networks and cellular energetics, *J. Exp. Biol.* 206 (2003) 2039–2047.
- [61] P.P. Dzeja, R.J. Zeleznikar, N.D. Goldberg, Suppression of creatine kinase-catalyzed phosphotransfer results in increased phosphoryl transfer by adenylate kinase in intact skeletal muscle, *J. Biol. Chem.* 271 (1996) 12847–12851.
- [62] R.J. Zeleznikar, P.P. Dzeja, N.D. Goldberg, Adenylate kinase-catalyzed phosphoryl transfer couples ATP utilization with its generation by glycolysis in intact muscle, *J. Biol. Chem.* 270 (1995) 7311–7319.
- [63] R.J. Zeleznikar, R.A. Heyman, R.M. Graeff, T.F. Walseth, S.M. Dawis, E.A. Butz, N.D. Goldberg, Evidence for compartmentalized adenylate kinase catalysis serving a high energy phosphoryl transfer function in rat skeletal muscle, *J. Biol. Chem.* 265 (1990) 300–311.
- [64] P.P. Dzeja, R.J. Zeleznikar, N.D. Goldberg, Adenylate kinase: kinetic behavior in intact cells indicates it is integral to multiple cellular processes, *Mol. Cell. Biochem.* 184 (1998) 169–182.
- [65] V. Spirin, M.S. Gelfand, A.A. Mironov, L.A. Mirny, A metabolic network in the evolutionary context: multiscale structure and modularity, *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006) 8774–8779.
- [66] P. Dzeja, A. Terzic, Adenylate kinase and AMP signaling networks: metabolic monitoring, signal communication and body energy sensing, *Int. J. Mol. Sci.* 10 (2009) 1729–1772.
- [67] I.M. de la Fuente, Quantitative analysis of cellular metabolic dissipative, self-organized structures, *Int. J. Mol. Sci.* 11 (2010) 3540–3599.
- [68] H. Qian, Open-system nonequilibrium steady state: statistical thermodynamics, fluctuations, *J. Phys. Chem. B* 110 (2006) 15063–15074.
- [69] K. Tepp, I. Shevchuk, V. Chekulayev, N. Timohhina, A.V. Kuznetsov, R. Guzun, V. Saks, T. Kaambre, High efficiency of energy flux controls within Mitochondrial Interactomes in cardiac intracellular energetic units, *Biochim. Biophys. Acta* 1807 (12) (Dec 2011) 1549–1561.
- [70] R. Moreno-Sanchez, S. Devars, F. Lopez-Gomez, A. Uribe, N. Corona, Distribution of control of oxidative phosphorylation in mitochondria oxidizing NAD-linked substrates, *Biochim. Biophys. Acta* 1060 (1991) 284–292.
- [71] H.V. Westerhoff, A. Kolodkin, R. Conradie, S.J. Wilkinson, F.J. Bruggeman, K. Krab, H.J. van Schuppen, H. Hardin, B.M. Bakker, M.J. Mone, K.N. Rybakova, M. Eijken, H.J. van Leeuwen, J.L. Snoep, Systems biology towards life in silico: mathematics of the control of living cells, *J. Math. Biol.* 58 (2009) 7–34.

- [72] H. Honda, K. Tanaka, N. Akita, T. Haneda Cyclical, Cyclical changes in high-energy phosphates during the cardiac cycle by pacing-Gated 31P nuclear magnetic resonance, *Circ. J.* 66 (2002) 80–86.
- [73] M.K. Aliev, F.A. van Dorsten, M.G. Nederhoff, C.J. van Echteld, V. Veksler, K. Nicolay, V.A. Saks, Mathematical model of compartmentalized energy transfer: its use for analysis and interpretation of 31P-NMR studies of isolated heart of creatine kinase deficient mice, *Mol. Cell. Biochem.* 184 (1998) 209–229.
- [74] M. Vendelin, O. Kongas, V. Saks, Regulation of mitochondrial respiration in heart cells analyzed by reaction diffusion model of energy transfer, *Am. J. Physiol. Cell Physiol.* 278 (2000) C747–C764.
- [75] T. Anmann, R. Guzun, N. Beraud, S. Pelloux, A.V. Kuznetsov, L. Kogerman, T. Kaambre, P. Sikk, K. Paju, N. Peet, E. Seppet, C. Ojeda, Y. Tourneur, V. Saks, Different kinetics of the regulation of respiration in permeabilized cardiomyocytes and in HL-1 cardiac cells. Importance of cell structure/organization for respiration regulation, *Biochim. Biophys. Acta* 1757 (2006) 1597–1606.
- [76] C. Monge, N. Beraud, K. Tepp, S. Pelloux, S. Chahboun, T. Kaambre, L. Kadaja, M. Roosimaa, A. Piirsoo, Y. Tourneur, A.V. Kuznetsov, V. Saks, E. Seppet, Comparative analysis of the bioenergetics of adult cardiomyocytes and nonbeating HL-1 cells: respiratory chain activities, glycolytic enzyme profiles, and metabolic fluxes, *Can. J. Physiol. Pharmacol.* 87 (2009) 318–326.
- [77] S. Patra, S. Bera, S. SinhaRoy, S. Ghoshal, S. Ray, A. Basu, U. Schlattner, T. Wallimann, M. Ray, Progressive decrease of phosphocreatine, creatine and creatine kinase in skeletal muscle upon transformation to sarcoma, *FEBS J.* 275 (2008) 3236–3247.
- [78] S. Patra, A. Ghosh, S.S. Roy, S. Bera, M. Das, D. Talukdar, S. Ray, T. Wallimann, M. Ray, A short review on creatine-creatine kinase system in relation to cancer and some experimental results on creatine as adjuvant in cancer therapy, *Amino Acids*, doi:10.1007/s00726-011-0974-3 [2011 Jul 19, Electronic publication ahead of print].
- [79] M. Eimre, K. Paju, S. Pelloux, N. Beraud, M. Roosimaa, L. Kadaja, M. Gruno, N. Peet, E. Orlova, R. Remmelkoor, A. Piirsoo, V. Saks, E. Seppet, Distinct organization of energy metabolism in HL-1 cardiac cell line and cardiomyocytes, *Biochim. Biophys. Acta* 1777 (2008) 514–524.
- [80] E.N. Maldonado, J. Patnaik, M.R. Mullins, J.J. Lemasters, Free tubulin modulates mitochondrial membrane potential in cancer cells, *Cancer Res.* 70 (24) (Dec 15 2010) 10192–10201.