J Bioenerg Biomembr (2013) 45:319–331
DOI 10.1007/s10863-012-9494-4

MINI-REVIEW

# Matters of the heart in bioenergetics: mitochondrial fusion into continuous reticulum is not needed for maximal respiratory activity

**Minna Varikmaa · Rita Guzun · Alexei Grichine · Marcela Gonzalez-Granillo · Yves Usson · François Boucher · Tuuli Kaambre · Valdur Saks**

Published online: 28 December 2012


**Abstract** Mitochondria are dynamic structures for which fusion and fission are well characterized for rapidly dividing cells in culture. Based on these data, it has recently been proposed that high respiratory activity is the result of fusion and formation of mitochondrial reticulum, while fission results in fragmented mitochondria with low respiratory activity. In this work we test the validity of this new hypothesis by analyzing our own experimental data obtained in studies of isolated heart mitochondria, permeabilized cells of cardiac phenotype with different mitochondrial arrangement and dynamics. Additionally, we reviewed published data including electron tomographic investigation of mitochondrial membrane-associated structures in heart cells. Oxygraphic studies show that maximal ADP-dependent respiration rates are equally high both in isolated heart mitochondria and in permeabilized cardiomyocytes. On the contrary, these rates are three times lower in NB HL-1 cells with fused mitochondrial reticulum. Confocal and electron tomographic studies show that there is no mitochondrial reticulum in cardiac cells, known to contain 5,000–10,000 individual, single mitochondria, which are regularly arranged at the level of sarcomeres and are at Z-lines separated from each other by membrane structures, including the T-tubular system in close connection to the sarcoplasmic reticulum. The new structural data in the literature show a principal role for the elaborated T-tubular system in organization of cell metabolism by supplying calcium, oxygen and substrates from the extracellular medium into local domains of the cardiac cells for calcium cycling within Calcium Release Units, associated with respiration and its regulation in Intracellular Energetic Units.

**Keywords** Cardiac cells · Respiration · Mitochondria · Fusion-fission · Calcium metabolism · Energy metabolism

M. Varikmaa · T. Kaambre · V. Saks
Laboratory of Bioenergetics, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia

R. Guzun · M. Gonzalez-Granillo · V. Saks
INSERM U1055, Laboratory of Fundamental and Applied Bioenergetics, Joseph Fourier University, Grenoble, France

A. Grichine · Y. Usson
Life Science Imaging–In Vitro Platform, IAB CRI U823 Inserm/ Joseph Fourier University, Grenoble, France

F. Boucher
Experimental, Theoretical and Applied Cardio-Respiratory Physiology, Laboratory TIMC-IMAG, Joseph Fourier University, Grenoble, France

V. Saks (✉)
Laboratory of Bioenergetics, Joseph Fourier University, 2280, Rue de la Piscine, BP53X-38041 Grenoble Cedex 9, France
e-mail: vsaks@ujf-grenoble.fr

## Introduction

Pioneering studies of Bereiter-Hahn about 20 years ago have shown that mitochondria are dynamic structures capable of changing rapidly their morphological pattern (Bereiter-Hahn 1990; Bereiter-Hahn and Voth 1994). With their work the authors opened the way to intensive studies of mitochondrial dynamics in living cells (Twig et al. 2008; Yaffe 1999; Westermann 2010, 2012; Dorn 2012; Kuznetsov et al. 2009; Chen and Chan 2005; Chen et al. 2010). The dynamic morphological changes entail remodeling processes of mitochondria through the fusion and fission phenomena. These opposite events are essential for development, apoptosis, and ageing (Westermann 2010; Dorn 2012). Fission events associated with major changes in mitochondrial electro-chemical membrane potential ($\Delta\psi_m$) generate functionally divergent daughters required for the removal of damaged and inactive organelles, while fusion

mixes the content of parent mitochondria through the rapid diffusion of matrix proteins, due to the slower migration of inner and outer membrane components. Therefore, mitochondrial dynamics has an impact on mitochondrial turnover. Mitochondrial fusion allows efficient mixing of mitochondrial content, and it generates extended mitochondrial networks. Mitochondrial fusion needs essential GTPases proteins, e.g. mitofusin 1 and 2 (Mfn1, Mfn2) and optic atrophy protein 1 (OPA1). In mammals, Mfn1 and Mfn2 are found in the mitochondrial outer membrane (MOM), while OPA1 in the intermembrane space closely associates with the mitochondrial inner membrane (MIM). It has been suggested that OPA1 plays a role in cristae maintenance and its activity is dependent on the bioenergetic state of mitochondria, i.e. mitochondrial membrane potential-dependent (Westermann 2010, 2012; Chen and Chan 2005; Chen et al. 2010). Mfns and OPA1 work together to promote mitochondrial fusion. In mitochondrial fusion, MOM and MIM should fuse simultaneously in order to maintain the organelle integrity. It was shown that some cell stressors can trigger increased mitochondrial fusion, called stress-induced mitochondrial hyperfusion, in an Mfn1- and Opa1-dependent manner (Chen and Chan 2005; Chen et al. 2012a).

These intensive studies of mitochondrial fusion and fission, carried out mostly in rapidly dividing cells in culture, have led many authors to conclude that fusion and fission have important bioenergetic consequences (Westermann 2012; Jezek and Plecita-Hlavata 2009). Jezek and Plecita-Hlavata have proposed that only a single mitochondrion consisting of a continuous mitochondrial reticulum exists in healthy intact cells in the absence of stress signaling (Jezek and Plecita-Hlavata 2009), and several authors have concluded that mitochondrial fusion is necessary for normal mitochondrial functioning (Hom and Sheu 2009; Parra et al. 2011). Thus, Westermann has proposed that mitochondrial morphology adapts depending on the respiratory activity (Westermann 2012). This model proposes that interconnected mitochondrial networks are frequently present in metabolically and respiratory active cells, whereas small and fragmented mitochondria are more prevalent in quiescent and respiratory inactive cells: when respiratory activity is low, fragmented mitochondria are the preferred morphological state, whereas under respiratory conditions mitochondria undergo fusion to allow spreading of metabolites and macromolecules throughout the entire compartment. Thus, it was suggested that mitochondrial fusion generates extended mitochondrial networks and allows efficient mixing of mitochondrial content that increase the respiratory activity (Westermann 2012). In particular, this has been thought to be important in muscle cells, including heart (Westermann 2012; Hom and Sheu 2009; Parra et al. 2011). The latter conclusion is based on the earlier works of Skulachev group (Bakeeva et al. 1978; Amchenkova et al. 1988; Skulachev 2001), describing the hypothesis that mitochondrial reticulum may be helpful in conducting the membrane potential from an oxygen-rich subsarcolemmal area into the cell interior (Skulachev 2001). It can be concluded from all these proposals that mitochondrial respiratory activity and thus energy fluxes in the cells in vivo may be regulated by fusion-fission events.

The aim of our work is to check the validity of this new and interesting fundamental hypothesis of cellular bioenergetics. We have taken the advantage of our long-time experience of quantitative studies of the mechanisms of respiration regulation of isolated heart mitochondria and permeabilized cells of cardiac phenotype with very different structures: adult cardiomyocytes with regularly arranged separate mitochondria and cancerous HL-1 cells with mitochondrial reticulum. Also, we have carefully analyzed published structural data on 3-dimensional organization of membrane systems in the heart cells, including T-tubular systems and those responsible for calcium recycling, and mitochondrial intracellular arrangement. These important structural data have revealed the principal role of elaborated T-tubular system in the organization of metabolism and mitochondrial arrangement in the cardiomyocytes, making possible the direct supply of oxygen, substrates and calcium into local areas of the cell (Hayashi et al. 2009; Nivala et al. 2012a; Soeller and Cannell 1999). All these experimental data show directly and unequivocally that mitochondrial fusion is not needed for maximal respiratory activity, which is seen in the heart in the absence of any fused mitochondrial reticulum.

## Results and discussion

### Oxygraphic measurements of respiration rates

Figure 1 shows the images of isolated heart mitochondria, cardiomyocytes and non-beating HL-1 cells prepared as described before (Saks et al. 1975, 1991; Pelloux et al. 2006; Anmann et al. 2006; Monge et al. 2009). First shown is an electron microscopy image (Fig. 1a) of isolated mitochondria from rat heart which consists of double membrane–bound spherical or bean-shaped organelles about 1 μm in diameter without any fusion (Hackenbrock 1966; Appaix et al. 2003). The other two preparations shown by confocal microscopy image are permeabilized adult rat cardiomyocytes (Fig. 1b) and cancerous NB HL-1 cells of the cardiac phenotype (Fig. 1c). The arrangement of intermyofibrillar mitochondria in cardiomyocytes is remarkably regular following the crystal-like pattern (Vendelin et al. 2005; Collins et al. 2002; Aon et al. 2003). Cancerous NB HL-1 cells of cardiac phenotype display a dense reticulum of fused elongated mitochondrial threads surrounding nuclei (Fig. 1c). NB HL-1 cells which were obtained from the

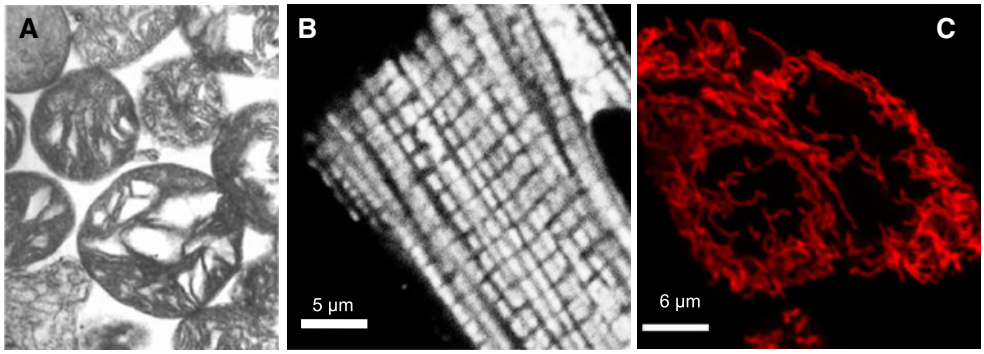


**Fig. 1** Different mitochondrial shape and arrangement in experimentally studied samples. **a** Electron microscopic image of isolated heart mitochondria (without fusion) (Appaix et al. 2003). **b** Confocal image of cardiomyocyte labeled with 20 nM MitoTracker Red (regularly arranged mitochondria) (Beraud et al. 2009). **c** Confocal image of NB HL-1 cells labeled with 20 nM MitoTracker Red (reticular fused mitochondria are seen) (Beraud et al. 2009)

mouse atrial cardiomyocyte tumor lineage are the type of cells with a clear fusion of mitochondria into reticular structures and altered energy metabolism (Pelloux et al. 2006; Anmann et al. 2006; Monge et al. 2009). Therefore, these cells are very useful as a reference system in structure-function relationship studies (Anmann et al. 2006).

Figure 2 shows recordings of oxygen consumption rates of isolated heart mitochondria (Fig. 2a), permeabilized

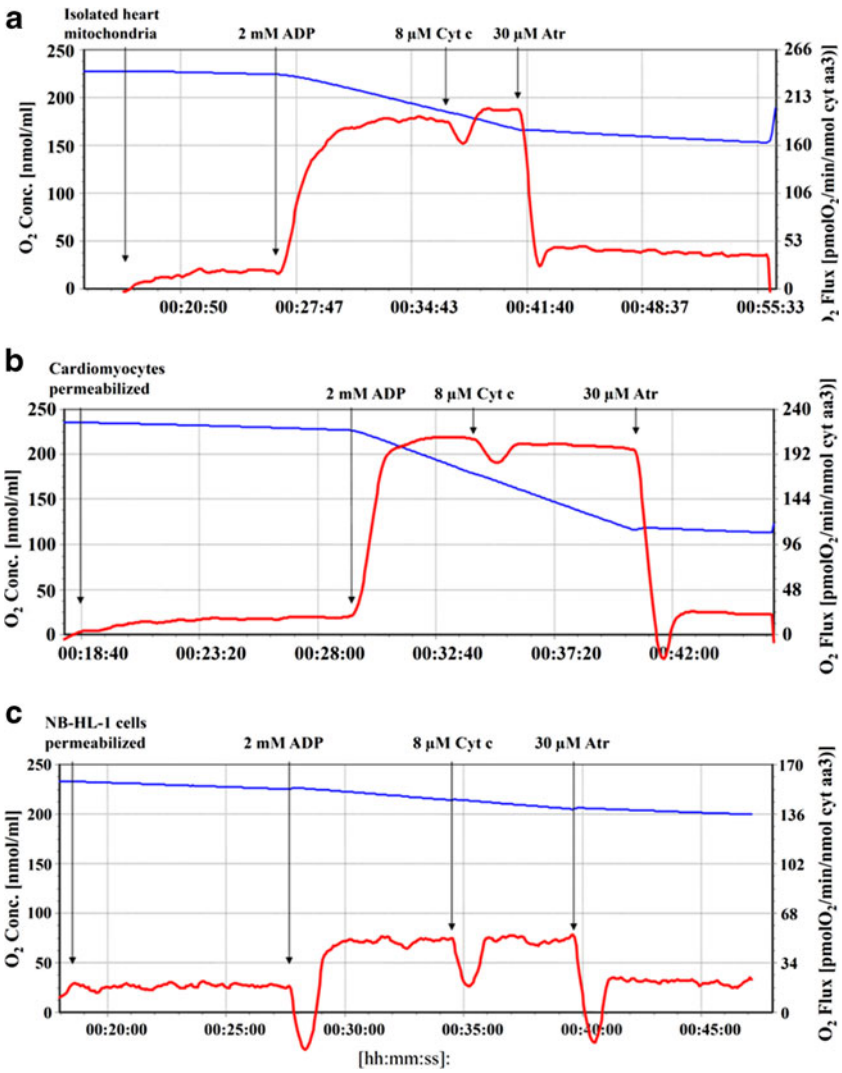


**Fig. 2** Respiratory parameters of different mitochondrial preparations. Respiratory control and maximal activity of isolated mitochondria (**a**), permeabilized cardiomyocytes (**b**) and NB HL-1 cells (**c**) were measured using oxygraphy (Oroboros, Austria) in Mitomed medium at 25 °C. *Blue line* and left axis show oxygen concentrations. *Red line* and right axis show oxygen flux which is recalculated per nmol cytochrome $aa_3$. Initial respiration rates ($V_0$) recorded in the presence of 5 mM glutamate and 2 mM malate correspond to State 2 of respiration. Maximal rate of respiration was measured by addition of 2 mM ADP. The addition of cytochrome c (Cyt c, 8 μM) did not change the respiration, indicating that the outer membrane is intact. Atractyloside (Atr, 30 μM) results in a decrease in respiration back to $V_0$ due to the inhibition of adenine-dinucleotide translocase. Data adapted from references (Anmann et al. 2006; Appaix et al. 2003; Saks et al. 2012; Guzun et al. 2009)

cardiomyocytes (Fig. 2b) and NB HL-1 cells (Fig. 2c). Initially respiration was recorded in the presence of glutamate-malate (basal rate, $V_0$, State 2 of respiration (Nicholls and Ferguson 2002)). Previously, Mootha et al. (1997) experimentally found that glutamate-malate are the optimal substrates yielding respiratory rates of heart mitochondria as high as any other substrates (Mootha et al. 1997). The maximal rate of respiration (Vmax, State 3 of respiration) was activated by the addition of 2 mM ADP. The stable rate of respiration after the addition of 8 μM cytochrome c and the decrease of the respiration rate until V0 after the addition of 30 μM atractyloside (State 4 of respiration) indicate the integrity of outer and inner mitochondrial membranes.

Figure 3 shows the analysis of respiratory activity of all three preparations studied. Figure 3a shows respiration rates (V0 and Vmax) expressed in nmoles of $O_2$ consumed per min per nmoles of cytochrome $aa_3$ (Saks et al. 1975, 1991; Pelloux et al. 2006; Anmann et al. 2006; Monge et al. 2009; Appaix et al. 2003; Saks et al. 1993, 2010, 2012; Saks 2007; Guzun et al. 2009, 2011). The Respiratory Control Index (RCI) calculated as the ratio between maximal ADP-stimulated respiration and respiration measured in the absence of ADP (States 3 to 4 ratio) is about 10 for isolated mitochondria and permeabilized cardiac myocytes, and about 2 for NB HL-1cells. Figure 3b shows the apparent affinity of oxidative phosphorylation for free ADP for these three preparations. The values of apparent Km for ADP are very low for isolated heart mitochondria and NB HL-1 cells (7.9±1.6 μM and 8.6±1.5 μM correspondingly) and very high for permeabilized cardiomyocytes (370.8±30.6 μM) (Anmann et al. 2006; Saks et al. 2010, 2012).

Thus, our experiments as many other previous observations show that individual regularly arranged mitochondria of permeabilized cardiomyocytes and isolated heart mitochondria have similar very high maximal ADP-stimulated respiration rates (Saks et al. 1993, 2012; Saks 2007; Guzun et al. 2009, 2011). State 3 respiratory activity is measured as the maximum ADP-Pi-driven respiratory rate with saturating levels of oxygen and substrates (Nicholls and Ferguson 2002). Mootha et al. (1997) found that the maximum ADP-Pi driven respiratory rates of the dog heart measured at 37 °C in the absence of oxygen or blood flow limitations were 676±31 $nmolO_2\ min^{-1}$ nmol Cyt $a^{-1}$. The RCI (ratio of state 3 to state 4 of respiration) ranged in Mootha's experiments between 8 and 15 at 37 °C. Recalculation of our experimental results, accounting for differences in the temperature, gives the values of respiration rates similar to those found in Mootha's experiments (Mootha et al. 1997).

Kinetic analysis of respiration regulation by ADP revealed a significant difference between isolated mitochondria and mitochondria in situ surrounded by intracellular structures. The apparent Km for ADP is much higher for permeabilized cardiomyocytes than for isolated mitochondria indicating the low availability of extramitochondrial ADP for the adenine nucleotide translocator (ANT) (Fig. 3b). It was shown that this effect is due to the limited selective permeability of MOM voltage-dependent anion channel (VDAC) for adenine nucleotides (Saks et al. 2010, 2012; Guzun et al. 2009; Rostovtseva et al. 2008; Gonzalez-Granillo et al. 2012) which can be increased by trypsin proteolysis of mitochondrial interactions with cytoskeletal proteins (Gonzalez-Granillo et al. 2012). The NB HL-1 cells display very high affinity for ADP and a very low maximal ADP-stimulated respiration rate (Fig. 3). Low values of apparent Km for ADP (about 8 μM) situated in the range of intracellular ADP concentration (about 50 μM) reflect a glycolytic rather than an oxidative metabolic pattern characteristic of rapidly proliferating immortalized cell lines,

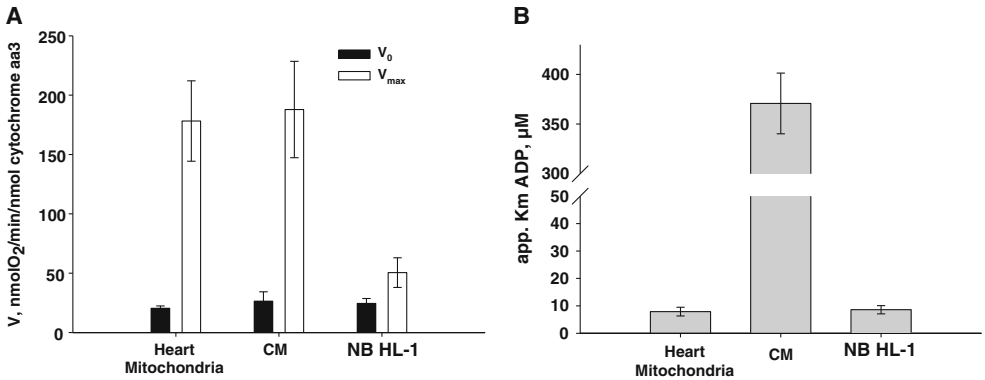


**Fig. 3** Maximal respiratory activities and kinetic analysis of ADP-stimulated respiration. **a** similar Vmax rates of isolated mitochondria and mitochondria in situ in permeabilized cardiac muscle cells. The Vmax of NB HL-1 cells is 4 fold lower. **b** The apparent affinity of oxidative phosphorylation for ADP of permeabilized adult cardiomyocytes is much lower (app. Km for ADP is 370.8±30.57 μM) than that of isolated mitochondria and NB HL-1 cells for ADP (7.9±1.6 μM and 8.6±1.5 μM). Data adapted from references (Anmann et al. 2006; Monge et al. 2009; Appaix et al. 2003; Saks et al. 1993, 2010, 2012; Saks 2007; Guzun et al. 2009, 2011)

cancerous and embryonic cells. Mitochondria of these cells usually form a reticular dynamic network. These experiments confirm that mitochondrial organization and function are cell type- and tissue-specific, and have to be studied separately for every type of cell. Chen et al. (2005) studied the relationship between mitochondrial organization and respiration activity using only one cell type, mouse embryonic fibroblasts (MEFs) displaying a different mitochondrial distribution due to the down- or up-regulation of fusion/fission proteins. Interestingly, cells overexpressing OPA1 with uniform small mitochondrial spheres spread throughout the cytoplasm had similar respiration rates with wild-type MEF cells (Chen et al. 2005). Cells under expressing OPA1 or lacking both Mfn1 and Mfn2 showed high heterogeneity in mitochondrial size. Some were very large mitochondrial spheres (several microns in diameter) accompanied by a scattering of very small mitochondrial fragments. These cells displayed pathological behavior with very slow growth, widespread heterogeneity of mitochondrial membrane potential, and decreased cellular respiration (Chen et al. 2005).

The conclusion from the first part of this study is that mitochondrial reticulum is not needed for maximal mitochondrial respiratory activity in the cells of a cardiac phenotype. On the contrary, mitochondrial fusion into reticular structures is seen in the pathological state and results in a decrease of respiratory capacity. The next important question is whether the mitochondrial reticular network exists in intact cardiomyocytes or not, and whether this structure is even possible. This question is analyzed in detail below.

## Confocal microscopic studies

Figure 4a shows the confocal image of auto-fluorescent mitochondrial flavoproteins (green color) in fixed adult cardiomyocytes and immuno-fluorescent labeling of α-actinin (red color) used to visualize Z-lines. Figure 4b shows analysis of fluorescence intensities along the line drawn through the length of sarcomeres. The localization of intensity peaks indicates the regular distribution of separated mitochondria between Z-lines of each sarcomere without any fusion into reticular structures. Only very rarely interconnections of

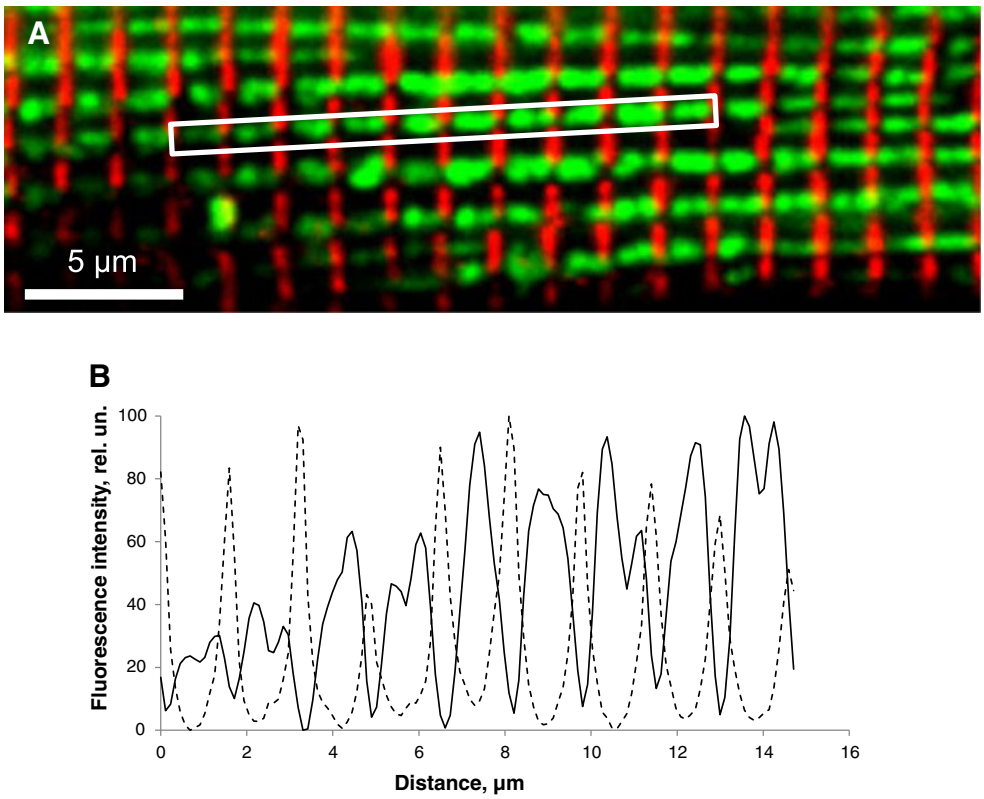


**Fig. 4** Mitochondrial and alpha-actinin distribution in adult rat cardiomyocyte. **a** Regular organization of individual mitochondria localized between Z-lines visualized by the autofluorescence of flavoproteines (*green color*). Z-lines are marked using immunofluorescent labeling of α-actinin (*red color*). **b** Analysis of fluorescence intensity along selected line in **a**. *Dotted line* fluorescence intensity line for actinine; *solid line* fluorescence intensity line for mitochondrial flavoproteins. Peaks of fluorescence intensity corresponding to mitochondria are seen in the regions of "zero" intensity of α-actinin indicating the absence of mitochondrial fusion into big reticulum. Only very rarely interconnection of mitochondrial fluorescence can be seen. Fluorescent images were acquired using a LSM710NLO confocal microscope (Carl Zeiss) equipped with a 100×/1.4 oil immersion plan-apochromat objective. Laser excitation was 488 nm for Dy light 488, and 633 nm for Cy5 and MitoTracker® Deep Red FM. Adapted with permission from reference (Gonzalez-Granillo et al. 2012)

mitochondrial fluorescence can be seen in Fig. 4. Earlier, studies of mitochondrial dynamics using high speed scanning (1 frame per 400 ms) confocal microscopy and gradient clustering algorithm revealed rapid and limited fluctuations of mitochondrial fluorescence centers which could correspond to the conformational changes of MIM (Beraud et al. 2009). No fusion-fission events of inter-myofibrillar mitochondria were observed in adult cardiomyocytes. Using the same technique, Beraud et al., in 2009 quantitatively described dynamic behavior of a mitochondrial network also in NB HL-1 cells (Beraud et al. 2009). Authors showed that mitochondria are continuously moving with the apparent flow velocity V of $6\times10$–4 μm/s when organelles are fragmented and about $2\times10$–4 μm/s when organelles are merged (Beraud et al. 2009). The high degree of freedom of mitochondria in NB HL-1 cells was explained by the lack of sarcomeres and the fusion-fission cycles (Beraud et al. 2009).

One of the important factors of the very regular arrangement of individual mitochondria in cardiomyocytes is their interaction with the cytoskeleton, in particular with tubulin (Saks et al. 2012; Guzun et al. 2011; Rostovtseva et al. 2008). Figure 5a shows the immunofluorescent labelling of VDAC of fixed cardiomyocyte mitochondria, and Fig. 5b shows the labeling of tubulin βII by its fluorescent antibodies in these cells. Remarkably, tubulin βII is found to be localized in close association with VDAC (Fig. 5c). After treatment of fixed cells at 95 °C mitochondria seem swollen in comparison with well separated mitochondria in nontreated intact cells (Fig. 4), but their positions and connection to tubulin βII are clearly seen. Biophysical measurements of VDAC permeability in vitro and kinetic analysis of regulation of mitochondrial respiration before and after addition of αβ-tubulin revealed that attachment of this protein to VDAC selectively decreases permeability of the latter for ATP and ADP (Rostovtseva et al. 2008; Monge et al. 2008). Additionally, we have shown that phosphocreatine (PCr) and creatine freely diffuse through MOM of isolated mitochondria (avoided of tubulin by trypsin treatment) as well as permeabilized cardiomyocytes which inherently display the co-localization of tubulin βII and mitochondria (Saks et al. 2010, 2012; Guzun et al. 2011; Timohhina et al. 2009). NB HL-1 cells, which are characterized by high affinity of oxidative phosphorylation for ADP (Km about 8 μM), do not express tubulin βII (Guzun et al. 2011). Other studied β-tubulin isotypes (I, III, IV) have a structural role with some particularities for cardiomyocytes and NB HL-1 cells. For instance, tubulin βIV is seen in polymerized form creating longitudinally and obliquely oriented microtubules in cardiac cells and filaments, radially distributed from nucleus to cell periphery, creating also inter-connections in NB HL-1 cells. Tubulin βIII is co-colocalized with sarcomeric Z-lines in cardiac cells and has a diffuse distribution penetrating into the nucleus in NB HL-1 cells. Tubulin βI has a similar diffuse distribution in both cell types (Guzun et al. 2011). It is important to mention that other cytoskeletal and cytoskeleton-associated proteins (plectin, kinesin, dynein, etc.) can also be involved in regulation of mitochondrial respiration and control MOM permeability for adenine metabolites.

## Analysis of structural data published in literature

Our conclusion from the results shown above is that there is still no direct evidence of the existence of a mitochondrial reticulum in heart cells. This conclusion is consistent with the results of very many earlier electron microscopic studies (Segretain et al. 1981; Fawcett and McNutt 1969; Hoppel et al. 2009) and recent observations by confocal microscopy

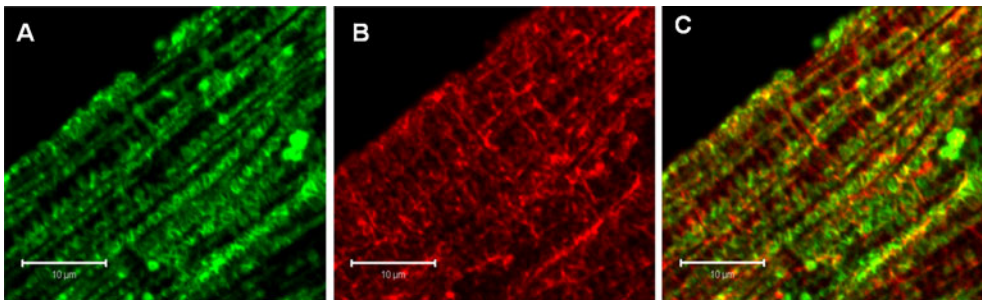


**Fig. 5** Simultaneous fluorescent immunolabeling of VDAC and βII tubulin in adult rat cardiomyocytes. **a** Fluorescent immunolabeling of VDAC (*green color*). After treatment of fixed cells at 95 °C mitochondria seem swollen but their positions are clearly seen. **b** Fluorescent immunolabeling of βII tubulin (*red color*) using mouse anti-tubulin βII (β2) and a Cy5 fluorescent secondary antibody. **c** Overlay of images A and B showing close localization of βII tubulin and VDAC. Scale bar: 10 μm. Freshly isolated cardiomyocytes were fixed in 4 % PFA at 37 °C for 15 min. After rinsing with PBS, cells were incubated in Antigen Retrieval Buffer (10 mM Tris, 5 % urea, pH 9.5) at 95 °C for 5 min, washed with PBS, permeabilized in 1 % Triton X-100 PBS solution at room temperature for 30 min, washed again with PBS, and blocked in PBS solution containing 2 % BSA for 30 min at 25 °C. Fixed and permeabilized cells were incubated overnight with rabbit anti-VDAC antibody (kindly provided by Dr. Catherine Brenner from University Paris-Sud, France). and with mouse anti-tubulin βII (Abcam, ab28036). The next day samples were labelled for 2 h at room temperature with two secondary antibodies DyLight 488 goat anti-rabbit IgG and DyLight549 goat anti-mouse IgG (Abcam, ab96899 and ab96880 correspondingly). Between staining steps samples were repeatedly rinsed with PBS

(Kuznetsov et al. 2009; Vendelin et al. 2005; Guzun et al. 2011). Most interestingly, however, the question of mitochondrial morphology and arrangement in cardiac cells was unequivocally and with absolute clarity solved in a recent excellent electron tomographic study by Hayashi et al. (2009) of membrane structures involved in calcium cycling in heart cells (Hayashi et al. 2009). This advanced technology achieved 5–8 nm resolution 3D microscopic analyses across multiple sarcomeres in mammalian cardiac muscle (Hayashi et al. 2009). This excellent breakthrough study and others (Nivala et al. 2012a; Soeller and Cannell 1999) have provided new data of principal importance which needs to be taken into account in checking the validity of the hypothesis that in muscle cells mitochondrial filaments connect a dense layer of mitochondria in the oxygen-rich cell periphery with mitochondria in the oxygen-poor core of the muscle fiber, thereby forming a continuous network to dissipate the membrane potential generated in the cell periphery over a large area and to use it to produce ATP in remote parts of the cell (Westermann 2010, 2012; Bakeeva et al. 1978; Amchenkova et al. 1988). The new structural data show very clearly that formation of a filamentous mitochondrial reticulum is not needed and even not possible in cardiac cells because of the fine spatial structure of extensions of sarcolemma into the cell interior, called T-tubular system, an elaborate network of transversal-axial tubules located at the level of Z-lines or between these lines of each sarcomere in close contact with sarcoplasmic reticulum and mitochondria, making possible the direct supply of calcium, substrates and oxygen from the extracellular medium into the cell to each mitochondria in these local areas, and also preventing mitochondrial fusion. This technique and information was not available when the original hypothesis was proposed (Bakeeva et al. 1978; Amchenkova et al. 1988; Skulachev 2001).

Because of the highest importance of these new data for understanding the relationship between cardiac cell structure, metabolism and mitochondrial arrangement, these data are discussed in detail below and most importantly the data are reproduced with the authors and publishers permissions.

### Calcium release units.

Calcium cycling is the mechanism of the excitation–contraction coupling which regulates the contraction of striated muscle cells (Bers 2001, 2002; Bers and Ginsburg 2007). In heart, a small amount of calcium enters into the cell from the extracellular medium during the depolarization phase of the action potential through the sarcolemmal calcium channels called also dyhydropyridine receptors (DHPR). Released is a larger amount of calcium from the sarcoplasmic reticulum (calcium induced calcium release, CICR) through the channels called ryanodine receptors (RyR). The calcium cycle is terminated by re-accumulation of calcium within the sarcoplasmic reticulum (SR) through the $Ca^{2+}$-dependent ATPase (SERCA) and part of calcium is exported into the extracellular medium via the Na-Ca exchanger in the sarcolemmal membrane (Bers and Ginsburg 2007). The elementary event in the calcium cycle is a calcium spark, a localized $Ca^{2+}$ released event due to random and collective opening of RyR channels clustered in the local areas called calcium release units, CRU. Each CRU contains about 10 sarcolemmal $Ca^{2+}$channels and several hundred RyR (Wang et al. 2004; Soeller et al. 2009; Nivala et al. 2011, 2012b). Different CRU form diffusively coupled networks in cardiomyocytes (Nivala et al. 2012b). The total number of CRUs in one cardiomyocyte has been found to be of the order of $10^4$ (Nivala et al. 2011, 2012b).

The calcium released in all diffusively connected CRUs activates sarcomere contraction by binding to the troponin complex on thin actin filaments making possible the myosin-actin interaction and thus contraction cycle (Bers 2001; Schneider et al. 2006). The force of contraction and cardiac work are regulated also by changes in sarcomere length (Schneider et al. 2006). This is the length-dependent mechanism of muscle contraction regulation, explaining the Frank-Starling law of the heart–the dependence of cardiac performance on left venticular filling (Saks et al. 2006a, 2012).

### Mitochondrial arrangement into Intracellular Energetic Units, ICEUs, and respiration regulation.

Mitochondria take part in the calcium cycle, importing calcium by a calcium uniport transporter and exporting it through the Na-Ca exchanger (Bers 2001; Glancy and Balaban 2012). In mitochondria, calcium is needed to maintain Krebs cycle dehydrogenases in an active state (Glancy and Balaban 2012). Mitochondrial respiration and ATP production are dependent both upon calcium entry into mitochondria from CRUs and metabolic signaling from myofibrillar ATPases in sarcomeres in which contraction is regulated by a Ca and sarcomere length-dependent mechanism (Saks et al. 2006a; Glancy and Balaban 2012).

About 50 % of DHPRs are located in the T-tubular system, which in the cells is localized transversely and axially (Hayashi et al. 2009; Soeller and Cannell 1999) close to Z-lines in the vicinity of junctional cisterns of SR (jSR) and mitochondria. Hayashi et al. (2009) studied the morphology and distribution of the T-tubular system, jSR and mitochondria in cardiomyocytes with the use of high resolution electron tomography. Most interestingly results of this excellent work are reproduced in Fig. 6. Figure 6a shows individual mitochondria localized at the level of A-band of sarcomeres. Figure 6b shows that at the Z-line mitochondria are in close contacts with the jSR and T-tubular system forming CRUs, which also separate mitochondria from each other, thus making their fusion impossible. At the level of

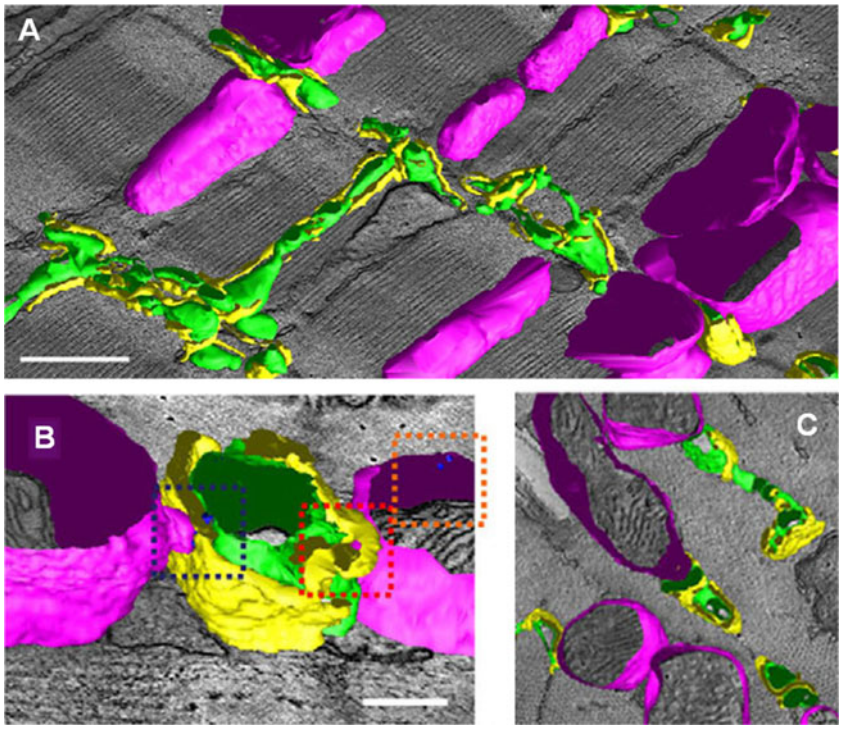


**Fig. 6** Electron tomographic imaging of mouse cardiac muscle. **a** Three-dimensional image of T-tubules (*green*), junctional sarcoplasmic reticulum (jSR in *yellow*), mitochondria (*magenta*). Scale bar: 1 μm. **b** Mitochondrial membrane-associated structures and T-tubules at larger magnification. Scale bar 200 nm. **c** The 3D mesh models of T-tubules, jSR and mitochondria in volume that crosscuts most of myofilaments. Scale bar 500 nm. Figures are reprinted with permission from Hayashi et al. (2009)

the sarcomere A-band one can see two smaller or one larger mitochondrion. Figure 6c shows the 3D image of separate mitochondria in another volume that crosscuts most of myofilaments. Again, one sees individual mitochondria separated from each other by cellular structures. These results are in concord with earlier 3D electron microscopic studies of cardiac cell structures by Segretain reproduced in Fig. 7 (Segretain et al. 1981). Figure 7a shows the crossection of cardiomyocyte at the H-band level where separate mitochondria are regularly surrounded by myofibrils. In a cross-section taken at the Z-line level no mitochondria are seen, only T-tubular system and SR (Fig. 7b). Figure 7c shows again that

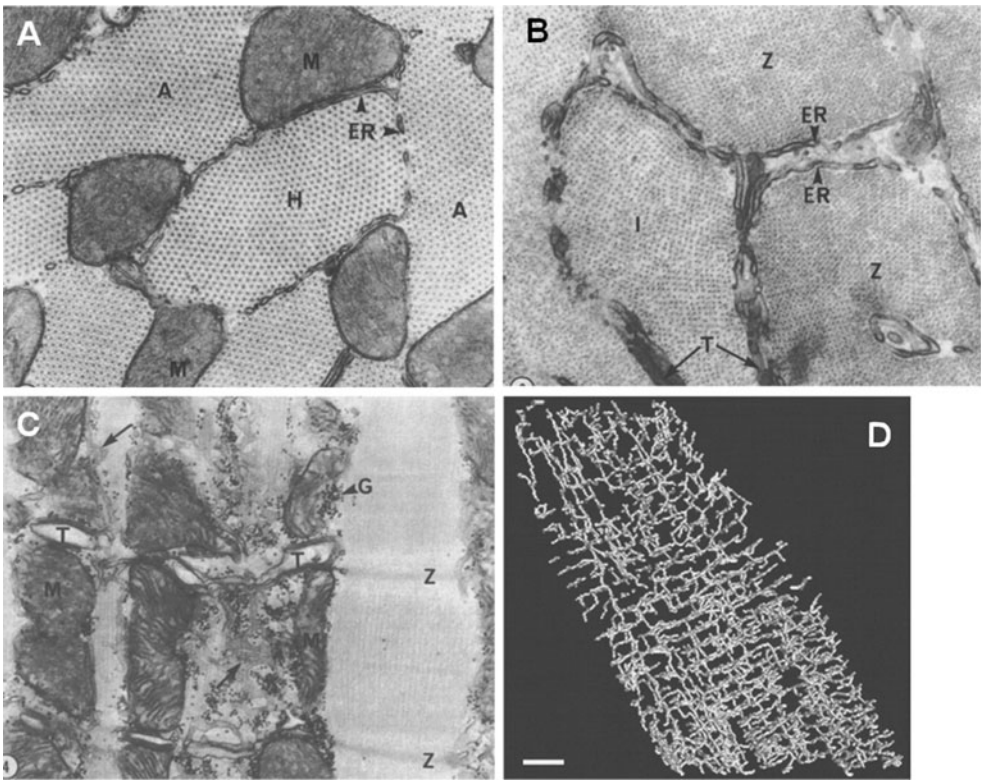


**Fig. 7** Electron microscopic imaging of cardiac muscle. The cross-section through muscle fiber shows **a**) at the H-band level (× 55.000) and **b**) at the Z-line level (× 70.000) mitochondrial membrane-associated structures constituted with endoplasmic reticulum (ER) and T-tubules. **c**) The longitudinal section through muscle fiber shows tubular cisternae (*arrows*) running transversely on each side of the T-tubule (T) at the level of Z-lines between two adjacent mitochondria. (G. : glycogen) × 33.000. Figure is reprinted with permission from Segretain et al. (1981). **d**) Three-dimensional skeleton of the T-tubular network in a rat ventricular myocyte reconstructed from a stack of fluorescence images. T-tubules create network mesh with some imposed regularity. Scale bar 55 μm. Figure is reprinted with permission from Soeller and Cannell (1999)

at the Z-line level mitochondria are separated by the T-tubular system and SR, those taking part in CRU.

Finally, Fig. 7d shows the 3D reconstruction of the T-tubular system in the cardiac cell (Soeller and Cannell 1999). This is the very elaborated and effective system for supplying $Ca^{2+}$, substrates and oxygen from the extracellular medium into cardiomyocytes. It plays an important role in cellular structural organization, metabolism and mitochondrial arrangement (Hayashi et al. 2009; Soeller and Cannell 1999). Discovery of this elaborated cellular architecture about 12 year ago profoundly changed our knowledge of heart cell structure and mechanisms of regulation of its metabolism (Soeller and Cannell 1999). These data show that the inter-myofibral mitochondria are at the same time subsarcolemmal ones in close contacts with the T-tubular system, and no distinction between these two populations is possible in the heart, in good agreement with the results of kinetic studies (Saks et al. 2012). Evidently, because of the very elaborated structure of the T-tubular system, all individual mitochondria have an equal and sufficient oxygen supply. Beraud et al. (2009) by using rapid scanning confocal microscopy showed that high frequency oscillations of the mitochondrial fluorescent centers occur only in the limited space between Z-lines. This is in concord with all structural data described above. It has also been shown in many laboratories that there is no electrical conductivity between individual mitochondria in cardiomyocytes (Kuznetsov et al. 2009; Timohhina et al. 2009; Nivala et al. 2011; Zorov et al. 2000; Collins and Bootman 2003; Zhou et al. 2012). Yaniv et al. (2011) measured simultaneously the sarcomere and mitochondrial dimensions in situ along the long-axis in isolated cardiomyocytes by registering variations in transmitted light intensity and directly observed mitochondria as micron-sized spheres localized between sarcomeres and distributed throughout the cell in a crystal-like lattice without any fusion. In this organized lattice, transient depolarizations of single mitochondria, known as flickers, due to ROS-induced opening of anion channels in the mitochondrial inner membranes may propagate in cells as depolarization waves (Nivala et al. 2011; Zhou et al. 2012). Cardiomyocytes were found to contain about 5,000–10,000 single mitochondria regularly arranged in the cell (Nivala et al. 2011).

All these data lead to the conclusion that the respiration of mitochondria is dependent on the events in the limited area of their localization in the vicinity of sarcomeres, SR and T-tubules. These structurally organized functional domains are called Intracellular Energetic Units, ICEUs (Saks et al. 2001, 2010, 2012) (Fig. 8). Due to elaborated structure of the T-tubular system, mitochondria in ICEUs are in contact both with myofibrils and sarcolemma, and therefore we cannot distinguish between subsarcolemmal or intermyofibrillar populations of mitochondria.

In the heart almost all ATP is synthesized by oxidative phosphorylation, and the linear relationship between heart workload (ATP hydrolysis) and oxygen consumption indicates very effective feedback regulation of mitochondrial respiratory activity in ICEUs (Appaix et al. 2003; Saks et al. 2006a, 2010, 2012). Furthermore, the large range of changes of ATP turnover rate that may increase 20 times from the resting state to maximal physiological workloads (Williamson et al. 1976), is seen in the presence of stable intracellular levels of such energy metabolites as the ADP, Pi, ATP and PCr (metabolic stability or homeostasis) (Williamson et al. 1976; Balaban 2002; Neely et al. 1972). Several mechanisms of regulation of mitochondrial ATP synthesis matching intracellular ATP needs were proposed to explain heart bioenergetics under conditions of metabolic stability. Among them are the above discussed Ca-dependent activation of Krebs cycle dehydrogenases (Glancy and Balaban 2012; Dorn and Maack 2012; Balaban 2002) and the control of oxidative phosphorylation on a beat to beat basis by the complex signal consisting of creatine/PCr ratio as well as the ADP, Pi and AMP small-scale local fluctuations within the contraction cycle (Saks et al. 2006a, 2010, 2012). It was demonstrated by Dzeja and Terzic groups using an $^{18}O$ tracer method that about 80 % of mitochondrial ATP is used for phosphocreatine (PCr) production in the mitochondrial creatine kinase (MtCK) reaction, the PCr flux carrying energy to all cites of ATP regeneration for ATPases within ICEUs (Fig. 8) (Dzeja and Terzic 2003; Dzeja et al. 2011). Metabolic control analysis of mitochondrial respiration regulation in cardiomyocytes has shown that by the mechanism of effective recycling of ADP-ATP in mitochondria, the MtCK reaction of PCr production coupled to oxidative phosphorylation is an effective amplifier of metabolic signals within ICEUs (Saks et al. 2012; Tepp et al. 2011). Glancy and Balaban (2012) have also recently concluded that "a possibility for the observed metabolic homeostasis in intact tissues is the compartmentation of metabolic intermediates in the cytosol much like that demonstrated for $Ca^{2+}$". The basic concept is that regional changes in ADP, Pi, and creatine in the regions around the mitochondria are major factors in driving mitochondrial ATP production.

Thus, matching of ATP synthesis to ATP hydrolysis for cellular work is the result of compartmentation of integrated metabolic processes, created by the interaction between cellular membranes, cytoskeletal proteins and organelles in the limited space of ICEUs (Saks et al. 2012). To overcome the restricted diffusion of metabolites in the cells with a high degree of structural organization, the most effective mechanism of functioning of organized metabolic pathways is the metabolic channeling of reaction intermediates within supercomplexes–metabolons (Beraud et al. 2009; Ovadi and Srere 2000; Saks et al. 2008, 2009; Wallimann et al. 1992). An example of such a processes is the intracellular

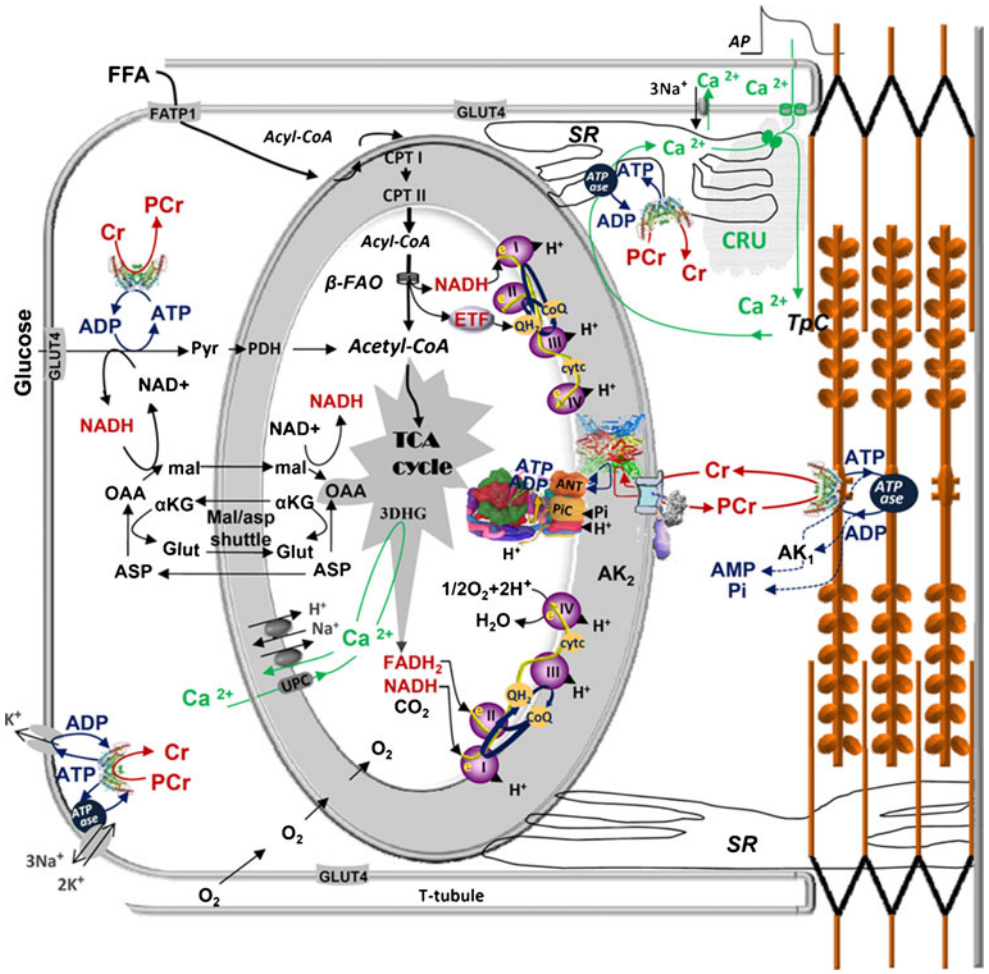


**Fig. 8** Functional scheme of the Intracellular Energetic Units of adult cardiac muscle cell. Mitochondria are structurally integrated with T-tubular network, sarcomere, and sarcoplasmic reticulum. Together with cytoskeletal proteins these structures create conditions for compartmentation of metabolic processes and calcium circulation. Substrates,Ca2+ and oxygen are delivered to mitochondria via the system of T-tubuls. Free fatty acids (FFA) taken up by a family of plasma membrane proteins (FATP1), are esterified to acyl-CoA which entering further the β-fatty acids oxidation (β-FAO) pathway which results in acetyl-CoA production. CPT I and CPT II—carnitine palmitoyltransferases I and II, respectively. 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 the mitochondrial inner membrane (MIM). Glucose (GLU) is taken up by glucose transporter-4 (GLUT-4) located both in the sarcolemma and T-tubules, and oxidized via the Embden–Meyerhof pathway. Pyruvate produced from glucose oxidation is transformed by the pyruvate dehydrogenise complex (PDH) into acetyl-CoA. The NADH redox potential resulting from glycolysis enters the mitochondrial matrix via malate–aspartate shuttle. Malate generated in the cytosol enters the matrix in exchange for α-ketoglutarate (αKG) and can be used to produce matrix NADH. Matrix oxaloacetate (OAA) is returned to the cytosol by conversion to aspartate (ASP) and exchange with glutamate (Glut). Acetyl-CoA is oxidized to CO2 in the tricarboxylic acids (TCA) cycle generating NADH and FADH2 which are further oxidized in the RC (complexes I, II) with final ATP synthesis. G6P inhibits HK decreasing the rate of glycolysis. The key system in energy transfer from mitochondria to cytoplasm is Mitochondrial Interactosome (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 βII-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 the MMCK reaction increases the Cr/PCr ratio which is transferred towards MtCK via CK/PCr shuttle. A small part of ADP resulting from ATP hydrolysis creates a gradient of concentration transmitted towards the matrix. The *shaded area* in the upper right corner shows the Calcium Release Unit. Calcium liberated from local intracellular stores during excitation–contraction coupling through a calcium-induced calcium release mechanism, (1) activates the contraction cycle by binding to troponin C in the troponin–tropomyosin complex of thin filaments and (2) enters the mitochondria mainly via the mitochondrial Ca2+ uniporter (UPC) to activate 3 Krebs cycle dehydrogenases: PDH, αKG, isocitrate dehydrogenase. Figure is reprinted with permission from Saks et al. (2012). Art work of the ATP synthasome in this Figure was reproduced with kind permission from P.L. Pedersen in part from Fig. 1 of reference (Pedersen 2008) and Fig. 2 of reference (Pedersen 2007) and is the result of the combined efforts of Drs. Young H. Ko and David J. Blum

glycolytic metabolon, mitochondrial TCA metabolon, β-fatty acids oxidation complex in the mitochondrial matrix, PDH complex (Saks 2007; Ovadi and Srere 2000) and even the formation of electron transport chain supercomplexes (Lenaz and Genova 2012). These reactions occur in ICEUs which are closely associated with CRUs (Saks et al. 2012) (Fig. 8). A mitochondrial substrate such as pyruvate (final product of anaerobic glycolysis) is directly transferred through the integrated MIM PDH enzyme complex giving acetyl CoA. The transfer of fatty acids towards the matrix is also dependent on the membrane enzymes: acetyl coenzyme-A synthetase and carnitine palmitoyl transferase (CPT1) in the outer, and CPT2 in the inner membrane (Saks et al. 2006b). Released into the matrix acyl coenzyme-A enters directly the β-fatty acids oxidation (β-FAO) pathway giving acetyl CoA. Once acetyl CoA is formed, the Krebs cycle begins. The Krebs cycle is a big metabolon attached to MIM and forms eight steps of enzymatic transformation of acetyl into $NADH,H^+$, $FADH_2$ and $CO_2$. All attempts to disrupt this metabolon will slow down formation of the reducing equivalents for the respiratory chain (Saks et al. 2006b). The electron transfer complexes have a tendency to form supercomplexes (Lenaz and Genova 2012; Acin-Perez et al. 2008), and it was also shown in Pedersen's laboratory (Ko et al. 2003) that ATP Synthase, Adenine Nucleotide Translocase (ANT) and the Phosphate Carrier (PIC) are incorporated into assemblies, called ATP Synthasomes.

Mixing the contents of all mitochondria into one reticulum may be expected only to destroy all these effective metabolic complexes, increasing diffusion time for intermediates and thus decreasing the energy fluxes (as seen in HL-1 cells), but not elevating the respiration rates (Westermann 2010, 2012).

Thus, if a fusion-fission cycle occurs in heart cells, it is a very infrequent phenomenon and does not include the formation of a continuous mitochondrial reticulum. It is not excluded that some fusion-fission may occur between two smaller or one larger mitochondria seen at the level of sarcomeres (Figs. 4 and 6), but the T-tubular system and cytoskeleton effectively prevent formation of a mitochondrial reticulum. It is also not excluded that some fusion may occur in the perinuclear mitochondrial clusters. Two main indirect pieces of evidence are used for mitochondrial fusion-fission dynamics in cardiac muscle cells (Dorn 2012). One is the presence of fusion-fission proteins in heart muscle and another consists in pathological remodeling of cardiac cells induced by abrogation of these proteins by genetic manipulations (Dorn 2012). However, fusion is not an exclusively mitochondrial feature. It is typical for such membranous intracellular structures as the sarcoplasmic reticulum, Golgi apparatus and intracellular vesicles. Koshiba's et al. (2004), have shown that mitochondrial fusions, like other intracellular membrane fusion events, proceeds through a tethering step mediated by a heptad repeat region (HR2). In normal adult cardiac cells fusion proteins may be tethering regularly arranged individual mitochondria to the surrounding membranous structures such as T-tubules and sarcoplasmic reticulum as shown in Scorrano's laboratory (de Brito and Scorrano 2008), rather than to induce fusion. In recent work, Chen et al. (2012b) have also shown that Mfn2 is essential for tethering mitochondria to sarcoplasmic reticulum. Similar data have been presented by Dorn and Maack (2012).

Moreover, the fusion-fission mitochondrial dynamics becomes evident for cardiac cells under pathological conditions such as ischemia-reperfusion (Dorn 2012). It is interesting that in all these situations mitochondrial fusion-fission is associated with the remodeling of sarcomeres and T-tubules. Cardiac failure in vivo due to the loss of fusongenic proteins associated with fragmentation of cardiac mitochondria into small heterogeneous conglomerates (Chen et al. 2012a) can be due to the disorganization of cell structure and metabolic compartmentation (remodeling of mitochondria-associated membrane interactions), impairment of intramitochondrial energy conversion, intracellular distribution of energy fluxes and controlling signals.

**Acknowledgments** This work was supported by INSERM and Agence Nationale de la Recherche programme SYBECAR, France, by grant No. 7823 from the Estonian Science Foundation and by grant SF0180114Bs08 from Estonia Ministry of Education and Science. The skillful technical assistance by Igor Shevchuk, Vladimir Chekulaev and Maire Peitel is gratefully acknowledged.

## References

Acin-Perez R, Fernandez-Silva P, Peleato ML, Perez-Martos A, Enriquez JA (2008) Respiratory active mitochondrial supercomplexes. Mol Cell 32:529–539

Amchenkova AA, Bakeeva LE, Chentsov YS, Skulachev VP, Zorov DB (1988) Coupling membranes as energy-transmitting cables. I. Filamentous mitochondria in fibroblasts and mitochondrial clusters in cardiomyocytes. J Cell Biol 107:481–495

Anmann T, Guzun R, Beraud N, Pelloux S, Kuznetsov AV, Kogerman L, Kaambre T, Sikk P, Paju K, Peet N, Seppet E, Ojeda C, Tourneur Y, Saks V (2006) 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 1757:1597–1606

Aon MA, Cortassa S, Marbán E, O'Rourke B (2003) Synchronized whole cell oscillations in mitochondrial metabolism triggered by a local release of reactive oxygen species in cardiac myocytes. J Biol Chem 278:44735–44744

Appaix F, Kuznetsov AV, Usson Y, Kay L, Andrienko T, Olivares J, Kaambre T, Sikk P, Margreiter R, Saks V (2003) Possible role of cytoskeleton in intracellular arrangement and regulation of mitochondria. Exp Physiol 88:175–190

Bakeeva LE, Chentsov YS, Skulachev VP (1978) Mitochondrial framework (reticulum mitochondriale) in rat diaphragm muscle. Biochim Biophys Acta 501:349–369

Balaban RS (2002) Cardiac energy metabolism homeostasis: role of cytosolic calcium. J Mol Cell Cardiol 34:1259–1271

Beraud N, Pelloux S, Usson Y, Kuznetsov AV, Ronot X, Tourneur Y, Saks V (2009) 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 41:195–214

Bereiter-Hahn J (1990) Behavior of mitochondria in the living cell. Int Rev Cytol 122:1–63

Bereiter-Hahn J, Voth M (1994) Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. Microsc Res Tech 27:198–219

Bers D (2001) Excitation-contraction coupling and cardiac contractile force, 2nd edn. Kluwer Academic Publishers, Dordrecht

Bers DM (2002) Cardiac excitation-contraction coupling. Nature 415:198–205

Bers DM, Ginsburg KS (2007) Na:Ca stoichiometry and cytosolic Ca-dependent activation of NCX in intact cardiomyocytes. Ann N Y Acad Sci 1099:326–338

Chen H, Chan DC (2005) Emerging functions of mammalian mitochondrial fusion and fission. Hum Mol Genet 14(Spec No. 2): R283–R289

Chen H, Chomyn A, Chan DC (2005) Disruption of fusion results in mitochondrial heterogeneity and dysfunction. J Biol Chem 280:26185–26192

Chen H, Vermulst M, Wang YE, Chomyn A, Prolla TA, McCaffery JM, Chan DC (2010) Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. Cell 141:280–289

Chen Y, Liu Y, Dorn GW 2nd (2012a) Mitochondrial fusion is essential for organelle function and cardiac homeostasis. Circ Res 109:1327–1331

Chen Y, Csordas G, Jowdy C, Schneider TG, Csordas N, Wang W, Liu Y, Kohlhaas M, Meiser M, Bergem S, Nerbonne JM, Dorn GW, Maack C (2012b) Mitofusin 2-containing mitochondrial-reticular microdomains direct rapid cardiomyocyte bioenergetic responses via inter-organelle Ca $^{2+}$ cross-talk. Circ Res 111(2012):863–875

Collins TJ, Bootman MD (2003) Mitochondria are morphologically heterogeneous within cells. J Exp Biol 206:1993–2000

Collins TJ, Berridge MJ, Lipp P, Bootman MD (2002) Mitochondria are morphologically and functionally heterogeneous within cells. EMBO J 21:1616–1627

de Brito OM, Scorrano L (2008) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature 456:605–610

Dorn GW 2nd (2012) Mitochondrial dynamics in heart disease. Biochim Biophys Acta http://dx.doi.org/10.1016/j.bbamcr.2012.03.008

Dorn GW 2nd, Maack C (2012) SR and mitochondria: calcium cross-talk between kissing cousins. J Mol Cell Cardiol doi.org/10.1016/j.yjmcc.2012.07.015

Dzeja PP, Terzic A (2003) Phosphotransfer networks and cellular energetics. J Exp Biol 206:2039–2047

Dzeja PP, Hoyer K, Tian R, Zhang S, Nemutlu E, Spindler M, Ingwall JS (2011) Rearrangement of energetic and substrate utilization networks compensate for chronic myocardial creatine kinase deficiency. J Physiol 589(5):193–211

Fawcett DW, McNutt NS (1969) The ultrastructure of the cat myocardium. I. Ventricular papillary muscle. J Cell Biol 42:1–45

Glancy B, Balaban RS (2012) Role of mitochondrial Ca $^{2+}$ in the regulation of cellular energetics. Biochemistry 51:2959–2973

Gonzalez-Granillo M, Grichine A, Guzun R, Usson Y, Tepp K, Chekulayev V, Shevchuk I, Karu-Varikmaa M, Kuznetsov AV, Grimm M, Saks V, Kaambre T (2012) 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 52:437–447

Guzun R, Timohhina N, Tepp K, Monge C, Kaambre T, Sikk P, Kuznetsov AV, Pison C, Saks V (2009) Regulation of respiration controlled by mitochondrial creatine kinase in permeabilized cardiac cells in situ. Importance of system level properties. Biochim Biophys Acta 1787:1089–1105

Guzun R, Karu-Varikmaa M, Gonzalez-Granillo M, Kuznetsov AV, Michel L, Cottet-Rousselle C, Saaremae M, Kaambre T, Metsis M, Grimm M, Auffray C, Saks V (2011) Mitochondria-cytoskeleton interaction: distribution of beta-tubulins in cardiomyocytes and HL-1 cells. Biochim Biophys Acta 1807:458–469

Hackenbrock CR (1966) Ultrastructural bases for metabolically linked mechanical activity in mitochondria. I. Reversible ultrastructural changes with change in metabolic steady state in isolated liver mitochondria. J Cell Biol 30:269–297

Hayashi T, Martone ME, Yu Z, Thor A, Doi M, Holst MJ, Ellisman MH, Hoshijima M (2009) Three-dimensional electron microscopy reveals new details of membrane systems for Ca $^{2+}$ signaling in the heart. J Cell Sci 122:1005–1013

Hom J, Sheu SS (2009) Morphological dynamics of mitochondria—a special emphasis on cardiac muscle cells. J Mol Cell Cardiol 46:811–820

Hoppel CL, Tandler B, Fujioka H, Riva A (2009) Dynamic organization of mitochondria in human heart and in myocardial disease. Int J Biochem Cell Biol 41:1949–1956

Jezek P, Plecita-Hlavata L (2009) Mitochondrial reticulum network dynamics in relation to oxidative stress, redox regulation, and hypoxia. Int J Biochem Cell Biol 41:1790–1804

Ko YH, Delannoy M, Hullihen J, Chiu W, Pedersen PL (2003) 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 278:12305–12309

Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM, Chan DC (2004) Structural basis of mitochondrial tethering by mitofusin complexes. Science 305:858–862

Kuznetsov AV, Hermann M, Saks V, Hengster P, Margreiter R (2009) The cell-type specificity of mitochondrial dynamics. Int J Biochem Cell Biol 41:1928–1939

Lenaz G, Genova ML (2012) Supramolecular organisation of the mitochondrial respiratory chain: a new challenge for the mechanism and control of oxidative phosphorylation. Adv Exp Med Biol 748:107–144

Monge C, Beraud N, Kuznetsov AV, Rostovtseva T, Sackett D, Schlattner U, Vendelin M, Saks VA (2008) Regulation of respiration in brain mitochondria and synaptosomes: restrictions of ADP diffusion in situ, roles of tubulin, and mitochondrial creatine kinase. Mol Cell Biochem 318:147–165

Monge C, Beraud N, Tepp K, Pelloux S, Chahboun S, Kaambre T, Kadaja L, Roosimaa M, Piirsoo A, Tourneur Y, Kuznetsov AV, Saks V, Seppet E (2009) 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 87:318–326

Mootha VK, Arai AE, Balaban RS (1997) Maximum oxidative phosphorylation capacity of the mammalian heart. Am J Physiol 272: H769–H775

Neely JR, Denton RM, England PJ, Randle PJ (1972) The effects of increased heart work on the tricarboxylate cycle and its interactions with glycolysis in the perfused rat heart. Biochem J 128:147–159

Nicholls DG, Ferguson SJ (2002) Bioenergetics3. Academic, London–New York, p 72

Nivala M, Korge P, Nivala M, Weiss JN, Qu Z (2011) Linking flickering to waves and whole-cell oscillations in a mitochondrial network model. Biophys J 101:2102–2111

Nivala M, de Lange E, Rovetti R, Qu Z (2012a) Computational modeling and numerical methods for spatiotemporal calcium cycling in ventricular myocytes. Front Physiol 3:1–12

Nivala M, Ko CY, Nivala M, Weiss JN, Qu Z (2012b) Criticality in intracellular calcium signaling in cardiac myocytes. Biophys J 102:2433–2442

Ovadi J, Srere PA (2000) Macromolecular compartmentation and channeling. Int Rev Cytol 192:255–280

Parra V, Verdejo H, del Campo A, Pennanen C, Kuzmicic J, Iglewski M, Hill JA, Rothermel BA, Lavandero S (2011) The complex interplay between mitochondrial dynamics and cardiac metabolism. J Bioenerg Biomembr 43:47–51

Pedersen PL (2007) Transport ATPases into the year 2008: a brief overview related to types, structures, functions and roles in health and disease. J Bioenerg Biomembr 39:349–355

Pedersen PL (2008) Voltage dependent anion channels (VDACs): a brief introduction with a focus on the outer mitochondrial compartment's roles together with hexokinase-2 in the "Warburg effect" in cancer. J Bioenerg Biomembr 40:123–126

Pelloux S, Robillard J, Ferrera R, Bilbaut A, Ojeda C, Saks V, Ovize M, Tourneur Y (2006) Non-beating HL-1 cells for confocal microscopy: application to mitochondrial functions during cardiac preconditioning. Prog Biophys Mol Biol 90:270–298

Rostovtseva TK, Sheldon KL, Hassanzadeh E, Monge C, Saks V, Bezrukov SM, Sackett DL (2008) Tubulin binding blocks mitochondrial voltage-dependent anion channel and regulates respiration. Proc Natl Acad Sci U S A 105:18746–18751

Saks V (ed) (2007) Molecular system bioenergetics. Energy for life. Gmbh, Germany: Wiley-VCH: Weinheim

Saks VA, Chernousova GB, Gukovsky DE, Smirnov VN, Chazov EI (1975) Studies of energy transport in heart cells. Mitochondrial isoenzyme of creatine phosphokinase: kinetic properties and regulatory action of $Mg^{2+}$ ions. Eur J Biochem 57:273–290

Saks VA, Belikova YO, Kuznetsov AV (1991) In vivo regulation of mitochondrial respiration in cardiomyocytes: specific restrictions for intracellular diffusion of ADP. Biochim Biophys Acta 1074:302–311

Saks VA, Vasil'eva E, Belikova YO, Kuznetsov AV, Lyapina S, Petrova L (1993) Retarded diffusion of ADP in cardiomyocytes: possible role of mitochondrial outer membrane and creatine kinase in cellular regulation of oxidative phosphorylation. Biochim Biophys Acta 1144:134–148

Saks VA, Kaambre T, Sikk P, Eimre M, Orlova E, Paju K, Piirsoo A, Appaix F, Kay L, Regitz-Zagrosek V, Fleck E, Seppet E (2001) Intracellular energetic units in red muscle cells. Biochem J 356:643–657

Saks V, Dzeja P, Schlattner U, Vendelin M, Terzic A, Wallimann T (2006a) Cardiac system bioenergetics: metabolic basis of the Frank-Starling law. J Physiol 571:253–273

Saks V, Favier R, Guzun R, Schlattner U, Wallimann T (2006b) Molecular system bioenergetics: regulation of substrate supply in response to heart energy demands. J Physiol 577:769–777

Saks V, Beraud N, Wallimann T (2008) Metabolic compartmentation—a system level property of muscle cells: real problems of diffusion in living cells. Int J Mol Sci 9:751–767

Saks V, Monge C, Guzun R (2009) Philosophical basis and some historical aspects of systems biology: from Hegel to Noble–applications for bioenergetic research. Int J Mol Sci 10:1161–1192

Saks V, Guzun R, Timohhina N, Tepp K, Varikmaa M, Monge C, Beraud N, Kaambre T, Kuznetsov A, Kadaja L, Eimre M, Seppet E (2010) Structure-function relationships in feedback regulation of energy fluxes in vivo in health and disease: mitochondrial interactosome. Biochim Biophys Acta 1797:678–697

Saks V, Kuznetsov AV, Gonzalez-Granillo M, Tepp K, Timohhina N, Karu-Varikmaa M, Kaambre T, Dos Santos P, Boucher F, Guzun R (2012) Intracellular energetic units regulate metabolism in cardiac cells. J Mol Cell Cardiol 52:419–436

Schneider NS, Shimayoshi T, Amano A, Matsuda T (2006) Mechanism of the Frank-Starling law—a simulation study with a novel cardiac muscle contraction model that includes titin and troponin I. J Mol Cell Cardiol 41:522–536

Segretain D, Rambourg A, Clermont Y (1981) Three dimensional arrangement of mitochondria and endoplasmic reticulum in the heart muscle fiber of the rat. Anat Rec 200:139–151

Skulachev VP (2001) Mitochondrial filaments and clusters as intracellular power-transmitting cables. Trends Biochem Sci 26:23–29

Soeller C, Cannell MB (1999) Examination of the transverse tubular system in living cardiac rat myocytes by 2-photon microscopy and digital image-processing techniques. Circ Res 84:266–275

Soeller C, Jayasinghe ID, Li P, Holden AV, Cannell MB (2009) Three-dimensional high-resolution imaging of cardiac proteins to construct models of intracellular Ca2+ signalling in rat ventricular myocytes. Exp Physiol 94:496–508

Tepp K, Shevchuk I, Chekulayev V, Timohhina N, Kuznetsov AV, Guzun R, Saks V, Kaambre T (2011) High efficiency of energy flux controls within mitochondrial interactosome in cardiac intracellular energetic units. Biochim Biophys Acta 1807:1549–1561

Timohhina N, Guzun R, Tepp K, Monge C, Varikmaa M, Vija H, Sikk P, Kaambre T, Sackett D, Saks V (2009) Direct measurement of energy fluxes from mitochondria into cytoplasm in permeabilized cardiac cells in situ: some evidence for mitochondrial interactosome. J Bioenerg Biomembr 41:259–275

Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS (2008) Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J 27:433–446

Vendelin M, Beraud N, Guerrero K, Andrienko T, Kuznetsov AV, Olivares J, Kay L, Saks VA (2005) Mitochondrial regular arrangement in muscle cells: a "crystal-like" pattern. Am J Physiol Cell Physiol 288:C757–C767

Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM (1992) Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. Biochem J 281(Pt 1):21–40

Wang SQ, Wei C, Zhao G, Brochet DX, Shen J, Song LS, Wang W, Yang D, Cheng H (2004) Imaging microdomain Ca $^{2+}$ in muscle cells. Circ Res 94:1011–1022

Westermann B (2010) Mitochondrial fusion and fission in cell life and death. Nat Rev Mol Cell Biol 11:872–884

Westermann B (2012) Bioenergetic role of mitochondrial fusion and fission. Biochim Biophys Acta http://dx.doi.org/10.1016/j.bbabio.2012.02.033

Williamson JR, Ford C, Illingworth J, Safer B (1976) Coordination of citric acid cycle activity with electron transport flux. Circ Res 38: I39–I51

Yaffe MP (1999) The machinery of mitochondrial inheritance and behavior. Science 283:1493–1497

Yaniv Y, Juhaszova M, Wang S, Fishbein KW, Zorov DB, Sollott SJ (2011) Analysis of mitochondrial 3D-deformation in cardiomyocytes during active contraction reveals passive structural anisotropy of orthogonal short axes. PLoS One 6:e21985

Zhou L, Aon MA, Liu T, O'Rourke B (2012) Dynamic modulation of Ca2+ sparks by mitochondrial oscillations in isolated guinea pig cardiomyocytes under oxidative stress. J Mol Cell Cardiol 51:632–639

Zorov DB, Filburn CR, Klotz LO, Zweier JL, Sollott SJ (2000) Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. J Exp Med 192:1001–1014