Cardiovascular Research (2013) **98**, 259–268
doi:10.1093/cvr/cvt032

SPOTLIGHT REVIEW

Calcium release microdomains and mitochondria

Michael Kohlhaas and Christoph Maack*

Klinik für Innere Medizin III, Universitätsklinikum des Saarlandes, Homburg 66421, Germany

Received 12 December 2012; revised 6 February 2013; accepted 6 February 2013; online publish-ahead-of-print 14 February 2013

Abstract The processes of excitation–contraction (EC) coupling consume large amounts of energy that need to be replenished by oxidative phosphorylation in the mitochondria. Since Ca^{2+} activates key enzymes of the Krebs cycle in the mitochondrial matrix, it is important to understand the mechanisms and kinetics of mitochondrial Ca^{2+} uptake to delineate how in cardiac myocytes, energy supply is efficiently matched to demand. In recent years, the identification of various proteins involved in mitochondrial Ca^{2+} signalling and the tethering of mitochondria to the sarcoplasmic reticulum (SR) has considerably advanced the field and supported the concept of a mitochondrial Ca^{2+} microdomain, in which Ca^{2+} concentrations are high enough to overcome the low Ca^{2+} affinity of the principal mitochondrial Ca^{2+} uptake mechanism, the Ca^{2+} uniporter. Furthermore, defects in EC coupling that occur in heart failure disrupt SR-mitochondrial Ca^{2+} crosstalk and may cause energetic deficit and oxidative stress, both factors that are thought to be causally involved in the initiation and progression of the disease.

Keywords Calcium • Mitochondria • Microdomain • Redox • Heart failure

The article is part of the Spotlight Issue on: T-tubules and ryanodine receptor microdomain signalling in cardiac hypertrophy and failure.

1. Introduction

Excitation–contraction (EC) coupling in cardiac myocytes consumes vast amounts of energy in the form of ATP that need to be efficiently replenished by oxidative phosphorylation in the mitochondria, which occupy roughly a third of cell volume. During a single heartbeat, ~2% of the cellular ATP is consumed, and the whole ATP pool of cardiac myocytes is turned over within less than a minute.[1–3] To orchestrate oxidative phosphorylation in response to constantly changing workloads of the heart, various factors tightly control the mitochondrial redox state and electron flux along the respiratory chain, securing constant availability of ATP. Two of the most important regulatory factors in this regard are Ca^{2+} and ADP,[4–7] whose communication between different organelles and/or compartments within cardiac myocytes is organized by 'microdomains'.[8,9] Spatial regulation by microdomains is a particular requirement for signalling efficacy considering the highly organized architecture of cardiac myocytes, in which mitochondria comprise a third of the cell volume and are aligned regularly along the ATP-consuming myofilaments, tightly connected to the Ca^{2+} stores (i.e. the sarcoplasmic reticulum, SR) and strategically positioned in vicinity to the 'dyads', where activation of L-type Ca^{2+} channels (LTCC) triggers Ca^{2+} release from the junctional SR in a process coined 'Ca^{2+}-induced Ca^{2+} release'.

In the last couple of years, considerable progress has been made regarding the identification of the molecular nature and functional roles of various components involved in the transmission of Ca^{2+} between the SR and mitochondria. In particular, the identification of the molecular identities and functional roles of various proteins in the inner mitochondrial membrane (IMM) and outer mitochondrial membrane (OMM), but also the SR, has paved new avenues and spurred new enthusiasm in research on the mechanisms of mitochondrial Ca^{2+} uptake. Here, after giving a brief overview on EC coupling and mitochondrial energetics (for a more comprehensive review, see refs.[5,10]), we focus on these more recent findings with a special emphasis on the aspects relevant to cardiac myocytes and the pathophysiological changes that occur in chronic heart failure.

2. Excitation–contraction coupling

2.1 Physiology

During a cardiac action potential (AP), Ca^{2+} enters cardiac myocytes via LTCC, triggering an even greater release of Ca^{2+} from the SR via ryanodine receptors (RyR2), and this Ca^{2+} is available at the myofilaments to induce contraction. The amount of Ca^{2+} that entered the cell via LTCC is exported primarily via the sarcolemmal Na^+/Ca^{2+}

* Corresponding author: Tel: +49 6841 1621380; fax: +49 6841 1623434, Email: christoph.maack@uks.eu

exchanger (NCX) during diastole, whereas the Ca^{2+} that was released from the SR is taken back up by the SR Ca^{2+} ATPase (SERCA). In human, rabbit and guinea-pig cardiac myocytes, the distribution between SR-derived Ca^{2+} and LTCC-triggered Ca^{2+} influx is ~70/30, whereas in smaller animals (such as mice and rats), this is shifted towards an even higher contribution of the SR (~90/10).[10] The interplay between LTCC and RyR2 is facilitated by the close vicinity of both channels in the dyads between t-tubular sarcolemma and the junctional SR. The concept of such a Ca^{2+} microdomain is that for a brief period, the Ca^{2+} concentrations at the site of release (i.e. near the RyR2 or the LTCC) exceed the concentrations in the bulk cytosol of the cell by several orders of magnitude.[8,11] These Ca^{2+} peaks are limited spatially and temporarily by diffusion of Ca^{2+} away from these 'hot spots' to the rest of the cytosol, where Ca^{2+} binds to myofilaments to induce contraction. A similar microdomain exists between the SR and mitochondria (*Figure 1* with inset), facilitating an efficient Ca^{2+} transfer between both organelles to match the energy produced in the mitochondria to the demand generated by Ca^{2+}-dependent processes of EC coupling. We will allude to this mitochondrial Ca^{2+} microdomain—the focus of this review—in more detail further below.

2.2 Pathophysiology

In chronic heart failure, defects of EC coupling underlie systolic and diastolic dysfunction.[5,12,13] A central defect in failing cardiac myocytes is the decreased Ca^{2+} load of the SR, which is related to decreased SERCA activity and a leak of the RyR2. Furthermore, the three-dimensional t-tubular structure that provides efficient coupling of the membrane potential to triggering SR Ca^{2+} release is disturbed in various models of heart failure, which induces spatio-temporal dyssynchrony of cytosolic Ca^{2+} transients.[14–16] Initially considered as an adaptive mechanism, the cytosolic Na^+-concentration ($[Na^+]_i$) is elevated in failing cardiac myocytes, presumably related to an activation of the 'late Na^+ current' (late I_{Na}) and/or increased activity of the Na^+/H^+ exchanger.[17–21] This elevation of $[Na^+]_i$, in particular in combination with up-regulation of the NCX protein, favours the reverse-mode of the NCX to import Ca^{2+} during the AP and to decrease Ca^{2+}-efflux during diastole.[17,19,22–25] Thus, it is generally perceived that this elevation in $[Na^+]_i$ partly compensates for decreased SR Ca^{2+} load and improves contractility in heart failure.[12,22–27] In this review, we will also discuss how the elevation of $[Na^+]_i$ has a negative impact on mitochondrial Ca^{2+} uptake and redox state.

A principal mechanism to regulate EC coupling is β-adrenergic stimulation, which signals through phosphorylation of Ca^{2+} handling proteins mediated by protein kinase A (PKA) and Ca^{2+}/Calmodulin-dependent protein kinase II (CaMKII).[28,29] In heart failure, chronic β-adrenergic stimulation with subsequent dysregulation of CaMKII- and PKA-regulated phosphorylation of target proteins substantially contributes to the maladaptive changes of EC coupling (e.g. the leak of RyR2 and activation of late I_{Na}).[28–30] Furthermore, an energetic deficit[31,32] and mitochondrial oxidative stress[33,34] are thought to play causative roles in the progression of heart failure. Over the past couple of years, we developed the concept that based on the tight interplay between EC coupling, mitochondrial redox state, and the regulation of reactive oxygen species (ROS) production, defects in EC coupling play a causal role for the development of energetic deficit and oxidative stress in heart failure (*Figure 1*).[35]

3. Regulation of mitochondrial energetics and redox state during EC coupling

3.1 Matching energy supply and demand

In the mitochondrial matrix, the Krebs cycle produces NADH and $FADH_2$, which fuel electrons into the electron transport chain (ETC) through complexes I and II (*Figure 1*). This induces sequential redox reactions along the complexes of the ETC that promote the translocation of protons (H^+) across the IMM, creating a proton gradient (ΔpH) and an electrical gradient (DYm) which constitute the proton motive force ($\Delta\mu_H$) which is utilized by the F_1F_o-ATP synthase to regenerate ATP from ADP. *In vivo*, cardiac workload is increased especially by β-adrenergic stimulation, increasing the rate and amplitude of cytosolic Ca^{2+} transients, which in turn accelerates ATP consumption by Ca^{2+}-cycling proteins and the myofilaments. This pronounced ATP hydrolysis elevates the formation of ADP, stimulating ATP regeneration at the F_1F_o-ATP synthase. Since the F_1F_o-ATP synthase reaction is coupled to $\Delta\mu_H$, ATP production principally dissipates $\Delta\mu_H$. To maintain $\Delta\mu_H$, NADH donates more electrons to the ETC, which oxidizes NADH to NAD^+ (and $FADH_2$ to FAD). Since β-adrenergic stimulation elevates the rate and frequency of cytosolic Ca^{2+} transients, mitochondria accumulate Ca^{2+} through several uptake mechanisms,[36,37] but in particular, the mitochondrial Ca^{2+} uniporter (MCU; *Figure 1*).[38,39] In the mitochondrial matrix, Ca^{2+} stimulates key enzymes of the Krebs cycle to accelerate the regeneration of oxidized NAD^+ and FAD to reduced NADH and $FADH_2$.[4,7,40–46] Furthermore, Ca^{2+}-induced stimulation of the ETC, the F_1F_o-ATP synthase and the aspartate–glutamate shuttle further contribute to an acceleration of oxidative phosphorylation (for a recent review, see Glancy and Balaban[47]). Thus, Ca^{2+} and ADP regulate oxidative phosphorylation in a complimentary manner and secure relatively constant ratios of ATP/ADP,[48,49] and constant or even increasing ratios of $NADH/NAD^+$[50] during physiological variations of cardiac workload.

This complex interplay between ADP- and Ca^{2+}-induced regulation of oxidative phosphorylation and the mitochondrial redox state has been revealed by experiments in which *abrupt* changes of workload were provoked in isolated cardiac trabeculae,[4,40–42] cardiac myocytes[43,44] and/or computational modelling.[7,45,46] During more gradual escalations of workload, as they occur during β-adrenergic stimulation *in vivo*, these transitions of energetic intermediates are presumably less accentuated, reflecting the high efficiency of regulatory control mechanisms (i.e. Ca^{2+} and ADP) that quickly adapt the supply to the increased energy demand. Thus, given the central role of Ca^{2+} for regulating mitochondrial energetics, the mechanisms and kinetics of mitochondrial Ca^{2+} uptake are of utmost importance for our understanding of energy supply-and-demand matching in the heart.

3.2 Role of redox state for mitochondrial reactive oxygen species formation

In addition to its role for energy production, mitochondrial Ca^{2+} uptake also plays a key role in governing mitochondrial formation of ROS. It has been initially proposed that ~2% of O_2 consumption is aberrantly funnelled to superoxide (O_2-) production, however, these initial estimates were corrected to levels of ~0.2% more recently (for review, see Balaban *et al.*[51]). The major site of O_2- formation are complexes I and III of the ETC (*Figure 1*),[51,52] and

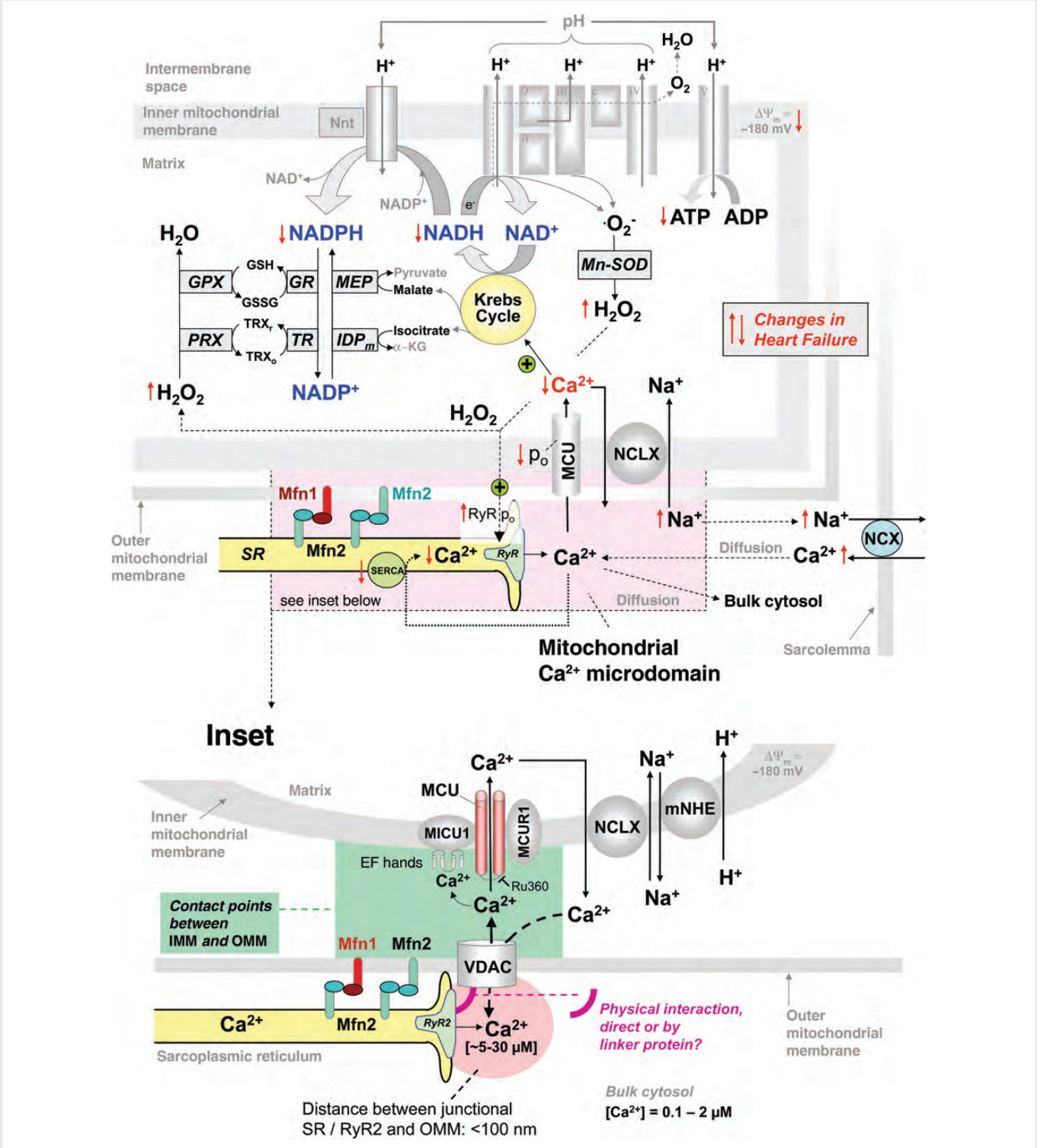

Figure 1 Mitochondrial Ca^{2+} signalling and its impact on mitochondrial energetics and redox state in health and disease. The Krebs cycle regenerates NADH required for oxidative phosphorylation at the electron transport chain, but also NADPH through isocitrate dehydrogenase (IDP_m), malic enzyme (MEP) and nicotinamide nucleotide transhydrogenase (Nnt). Ca^{2+} stimulates three key enzymes of the Krebs cycle and thus, regulates NADH and NADPH regeneration. In heart failure, defects in EC coupling and mitochondrial ion homoeostasis (indicated by red arrows) contribute to energetic mismatch and oxidative stress. $\Delta\Psi_m$, mitochondrial membrane potential; Mn-SOD, Mn^{2+}-dependent superoxide dismutase; PRX, peroxiredoxin; GPX, glutathione peroxidase; $TRX_{r/o}$, reduced/oxidized thioredoxin; GSH/GSSG, reduced/oxidized glutathione; TR, thioredoxin reductase; GR, glutathione reductase; α-KG, α-ketoglutarate; MCU, mitochondrial Ca^{2+} uniporter; NCLX, mitochondrial Na^+/Ca^{2+} (and Li^+) exchanger; p_o, open probability; RyR, ryanodine receptor; SERCA, SR Ca^{2+} ATPase; Mfn, mitofusin. Inset: detailed aspects of a mitochondrial Ca^{2+} microdomain. MICU1, mitochondrial Ca^{2+} uptake 1; mNHE, mitochondrial Na^+/H^+ exchanger; IMM and OMM, inner and outer mitochondrial membranes; VDAC, voltage-dependent anion channel.

O_2- formation is highest when substrates (glucose, fatty acids) are available, but energy (and thus, O_2) consumption are low, i.e. a condition that is experimentally defined as 'state 4 respiration'. In this situation, which typically does not occur in the heart where energy is constantly consumed, the ETC is highly reduced, and electrons are more likely to aberrantly 'slip' to O_2 to produce O_2-.[51,53] When workload increases, ADP-induced acceleration of respiration oxidizes the ETC by favouring the enzymatic reduction of O_2 to H_2O at complex IV (cytochrome oxidase), during which O_2- formation is efficiently avoided. At the same time, aberrant O_2- formation at complexes I and III is diminished.[51]

Besides the ETC, other mechanisms of mitochondrial ROS production have been proposed. Dedkova and Blatter[54] revealed that an uncoupled nNOS located in the mitochondria is a source for O_2- which can induce mPTP opening and cell death under pathological conditions of mitochondrial Ca^{2+} overload. The existence of a mitochondrial NOS, however, is still controversial (for review, see Brookes[55]). Furthermore, translocation of MAO[56] and NAPDH oxidase 4 (Nox4)[57,58] to mitochondria may contribute to mitochondrial oxidative stress. The respective contribution of these various potential sources to overall ROS production, however, requires further evaluation.

To protect the matrix enzymes and mitochondrial DNA from oxidative damage, antioxidative mechanisms are in place to eliminate ROS.[51,59] O_2- is rapidly transformed to H_2O_2 by superoxide dismutase (SOD), and H_2O_2 is detoxified by glutathione peroxidase (GPX) and peroxiredoxin (PRX; *Figure 1*). These H_2O_2-eliminating enzymes need to be regenerated by NADPH-dependent enzymes, such as glutathione reductase (GR) and thioredoxin reductase (TR). This assigns NADPH a central role in the terminal elimination of mitochondrial ROS.[59] NADPH is regenerated by three enzymes that all derive their substrates from products of the Krebs cycle, i.e. isocitrate dehydrogenase (IDP_m), malic enzyme and the nicotinamide nucleotide transhydrogenase (Nnt; *Figure 1*).[59] Since Ca^{2+} activates three rate-limiting enzymes of the Krebs cycle, we recently discovered that mitochondrial Ca^{2+} uptake is not only important to match energy supply to demand, but also to keep the redox state of NADPH in a reduced state to prevent overflow of H_2O_2.[44] During β-adrenergic stimulation, transient oxidation of NAD(P)H in response to ADP-induced acceleration of respiration was associated with a transient increase in mitochondrial H_2O_2 formation.[44] When blocking mitochondrial Ca^{2+} uptake with the MCU-inhibitor Ru360, the Ca^{2+}-induced (and Krebs cycle mediated) regeneration of NAD(P)H was blunted and the formation of H_2O_2 potentiated.[44]

Since RyRs are regulated by the cellular redox state,[60] a recent study by Liu and co-workers[61] revealed that variations of the mitochondrial NAD(P)H redox state and H_2O_2 production correlate with RyR2 activity, indexed by the frequency of elementary Ca^{2+} release events, i.e. Ca^{2+} sparks. These data were in agreement with a previous study that observed a bidirectional regulation of spark frequency by mitochondrial ROS production.[62] In this latter study, low ROS levels increased Ca^{2+} spark frequency, while higher levels suppressed them. Along similar lines, a recent study by Prosser *et al.*[63] linked stretch-induced increases in ROS production from an NADPH oxidase (Nox2) to increased spark frequency. Collectively, these studies suggest that moderate increases in ROS production activate RyRs and suggest a close interplay of mitochondrial and cytosolic redox state/ROS production and elementary Ca^{2+} release events, implying a redox feedback on EC coupling (*Figure 1*).

4. Mechanisms and kinetics of mitochondrial Ca^{2+} uptake and release

Although in recent years, considerable progress was made with regard to the molecular identity of proteins involved in mitochondrial Ca^{2+} uptake and release,[36–39,64–69] several questions still remain unanswered and some controversies persist.[5,70–72] As already addressed by Hüser *et al.*[73] in 2000, a central matter of debate was and is whether mitochondria take up Ca^{2+} rapidly, on a beat-to-beat basis, or rather slowly, integrating changes of amplitudes and frequency of cytosolic Ca^{2+} transients.[5,37] This controversy is based partly on different findings related to different techniques that can be applied to measure mitochondrial Ca^{2+} uptake in cardiac myocytes during EC coupling (for more comprehensive reviews, see refs.[5,71,74]). While most groups agree that beat-to-beat mitochondrial Ca^{2+} uptake can occur, the absolute quantities of mitochondrial Ca^{2+} fluxes during cytosolic Ca^{2+} transients remain incompletely resolved,[70,72] ranging from estimates of 10 nM/beat[75,76] to ~26% of the whole SR Ca^{2+} content during a cytosolic Ca^{2+} transient.[77]

4.1 Kinetics and molecular identity of the mitochondrial Ca^{2+} uniporter

Mitochondria take up Ca^{2+} primarily via the MCU, a recently identified 40-kDa channel protein in the IMM that is highly selective for Ca^{2+} (*Figure 1*).[38,39,78] In COS7 cells, the MCU has a high Ca^{2+} carrying capacity (5×10^6 Ca^{2+} molecules per second per single MCU molecule), and MCU density in the IMM (~10–40 per μm^2) is only slightly lower than that of voltage-gated Ca^{2+} channels in the sarcolemmal membrane (~100 per μm^2).[78,79] More recent evidence, however, showed that mouse heart mitochondria have dramatically lower MCU current densities than skeletal muscle mitochondria.[80] It is currently unclear whether this low current density is limited to the mouse with heart rates of ~600/min to avoid mitochondrial Ca^{2+} overload, or conserved across many species. The driving force for mitochondrial Ca^{2+} uptake is the large electrochemical gradient across the IMM ($\Delta\Psi_m$, ~ −180 mV), and early experiments on suspensions of isolated mitochondria revealed Ca^{2+} concentrations for half-maximal activation ($K_{0.5}$) of the MCU in the upper micromolar range.[81,82] In more recent patch-clamp-based studies, the $K_{0.5}$ was even higher in the millimolar range.[78,83] These Ca^{2+} concentrations are clearly beyond levels expected in the bulk cytoplasm during Ca^{2+} transients in myocytes (~1–2 μmol/L). In a study on human cardiac mitoplasts, two distinct mitochondrial Ca^{2+} channels were identified, coined mCa1 and mCa2, with mCa1 resembling the properties of the MCU and mCa2 with slightly different properties, possibly responsible for part of the non-Ru360-sensitive component of mitochondrial Ca^{2+} uptake.[83] Similarly, Wei *et al.*[84] identified two different modes of mitochondrial Ca^{2+} uptake with different sensitivities to Ru360, termed MCU_{mode1} and MCU_{mode2}. This will be discussed in more detail below.

The MCU is part of a macromolecular complex that also contains MICU1 (*Figure 1*, inset), the first protein involved in mitochondrial Ca^{2+} uptake whose molecular nature could be identified in HeLa cells by the Mootha group.[66] The identity of MICU1 was revealed by an integrative strategy that predicted human genes involved in mitochondrial Ca^{2+} uptake based on clues from comparative physiology, evolutionary genomics, and organelle proteomics.[66]

Systematic RNA interference experiments against 13 top candidates of this screen revealed MICU1 to be a protein that is associated with the IMM and that has two canonical EF hands that are required for its activity, indicating a role in Ca^{2+} sensing (*Figure 1*, inset).[66] Silencing MICU1 abolished mitochondrial Ca^{2+} uptake and attenuated metabolic coupling between cytosolic Ca^{2+} transients and activation of Krebs cycle dehydrogenases.[66] The observation that MICU1 overexpression did not enhance mitochondrial Ca^{2+} uptake and the fact that MICU1 has only one membrane-spanning domain, however, made it an unlikely candidate for the Ca^{2+} uniporter *per se*, but rather a regulatory protein that would act as a Ca^{2+} sensor based on the requirement of its two EF hands for proper function (see below).

In late 2011, two independent seminal papers from the Mootha and the Rizzuto groups unequivocally revealed the molecular identity of the Ca^{2+} uniporter.[38,39] Using comparable systematic computational screening approaches, a protein with two membrane-spanning domains that locates to the IMM was identified, now termed the MCU (*Figure 1*, inset). In contrast to MICU1, overexpression of MCU doubled mitochondrial Ca^{2+} uptake in HeLa cells,[39] while silencing MCU substantially reduced mitochondrial Ca^{2+} uptake in HeLa cells *in vitro*,[38,39] but also in liver mitochondria *in vivo*.[38] Reconstitution of the MCU in lipid bilayers yielded channel activity that resembled the electrophysiological properties and inhibitor sensitivity of the uniporter.[39] The MCU forms oligomers, physically interacts with MICU1 and is part of a multi-protein complex. According to both studies, a highly conserved linker between the two transmembrane domains that faces the intermembrane space is required for the inhibitory effect of Ru360 (*Figure 1*, inset), the most specific inhibitor of the MCU so far.[38,39] A recent study revealed that also in neonatal cardiac myocytes, silencing of the MCU reduced-, and overexpression potentiated mitochondrial Ca^{2+} uptake.[85]

Following up on MICU1,[66] whose exact modulatory role for mitochondrial Ca^{2+} uptake was initially unresolved, a recent study revealed an important physiological role of this protein.[65] Under resting conditions, mitochondrial Ca^{2+} levels are kept about five to six orders of magnitude lower than would be predicted by the electrochemical gradient across the IMM driven primarily by $\Delta\Psi_m$. In fact, earlier studies on isolated rat[86] and guinea-pig cardiac myocytes[43] had already observed a 'threshold' for mitochondrial Ca^{2+} uptake at cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_c$) of ~400–500 nmol/L; below this $[Ca^{2+}]_c$, $[Ca^{2+}]_m$ was $<[Ca^{2+}]_c$, while at higher values of $[Ca^{2+}]_c$, $[Ca^{2+}]_m$ rapidly increased.[86] The low Ca^{2+} affinity of the MCU is one explanation for this phenomenon,[78] restricting mitochondrial Ca^{2+} uptake to situations when $[Ca^{2+}]_c$ rises substantially. In this regard, MICU1 interacts with the MCU and sets a Ca^{2+} threshold for mitochondrial Ca^{2+} uptake without affecting the kinetic properties of MCU-mediated Ca^{2+} uptake *per se*.[65] Thus, MICU1 is considered a 'gatekeeper' of mitochondrial Ca^{2+} uptake that prevents mitochondrial Ca^{2+} overload,[65] which can lead to activation of the permeability transition pore (PTP) and subsequently, programmed cell death by necrosis (or potentially apoptosis).[87–89]

Another regulatory protein that binds to the MCU is MCUR1, an integral membrane protein required for MCU-dependent mitochondrial Ca^{2+} uptake.[69] MCUR1 knockdown abrogated mitochondrial Ca^{2+} uptake in intact and permeabilized cells and disrupted oxidative phosphorylation, which lowered cellular ATP and activated AMP kinase-dependent pro-survival autophagy. The roles of neither MCUR1 nor MICU1 have been addressed in cardiac myocytes yet.

Recently, the group of Anderson proposed an important regulatory role for CaMKII in regulating MCU activity.[90] They observed that CaMKII promoted opening of the mitochondrial PTP and myocardial death by enhancing the MCU current. A mitochondrial-targeted CaMKII-inhibitory protein reduced the MCU current, prevented mPTP opening (presumably by limiting mitochondrial Ca^{2+} overload after ischaemia/reperfusion), $\Delta\Psi_m$ dissipation, programmed cell death and infarct size to a similar extent as cyclosporine A, an inhibitor of the PTP.[90] Based on patch-clamp measurements, it was proposed that CaMKII regulates the MCU from the matrix side of the IMM.[90] Since CaMKII activity is increased by GPCR activation and oxidative stress, CaMKII-mediated regulation of MCU may resemble an attractive control mechanism of mitochondrial Ca^{2+} uptake. This concept, and in particular its relevance under physiological conditions, will require further investigation.

In addition to the MCU, several other mechanisms and/or channels for mitochondrial Ca^{2+} uptake have been proposed. Among them are the rapid mode of uptake (RaM), coenzyme Q10, RyR1, uncoupling proteins 2 and 3 and leucine-zipper-EF-hand-containing transmembrane protein 1 (Letm1). For a more comprehensive review on these Ca^{2+} uptake mechanisms, we refer the reader to two recent comprehensive reviews by O-Uchi *et al.*[36] and Dedkova and Blatter.[37]

4.2 Mitochondrial Ca^{2+} efflux

The primary mitochondrial Ca^{2+} efflux mechanism in cardiac myocytes is the mitochondrial NCX,[81,91] which is presumably electrogenic[92,93] by exchanging 1 Ca^{2+} for 3 Na^+, with a K_m for $[Na^+]_i$ of ~8 mM[94] and thus, within the physiological range of $[Na^+]_i$ in cardiac myocytes.[21,95] Recently, the molecular nature of the mitochondrial NCX was identified as well.[64] The exchanger forms dimers, locates to the mitochondrial cristae, transports either Na^+ or Li^+ in exchange for Ca^{2+} and thus, is termed NCLX (*Figure 1*).[64] Ca^{2+}-dependent mitochondrial Na^+ import via the NCLX is counterbalanced by mitochondrial Na^+/H^+ exchange (mNHE). This makes mitochondrial Ca^{2+} uptake an energetically expensive process, since $\Delta\mu_H$ is dissipated by the Na^+-dependent H^+ import to the matrix and needs to be regenerated by the ETC (*Figure 1*).

4.3 Integrative physiology of mitochondrial Ca^{2+} uptake and release in cardiac myocytes

While these studies are important to understand the molecular composition of the MCU with its regulatory proteins in general, they have not (yet) resolved the kinetics and quantities of mitochondrial Ca^{2+} uptake in cardiac myocytes during EC coupling. In the last couple of years, we have focused on this controversial issue by establishing a method by which cytosolic ($[Ca^{2+}]_c$) and mitochondrial Ca^{2+} concentrations ($[Ca^{2+}]_m$) are monitored in the same cell, respectively,[43] by loading myocytes with a cell-permeable Ca^{2+} indicator that—due to its positive charge—accumulates primarily in the mitochondrial matrix (the ester form of rhod-2, i.e. rhod-2/AM). To eliminate cytosolic traces of the dye, myocytes are patch-clamped and dialysed with a rhod-2-free pipette solution that contains indo-1 salt that locates only to the cytosol.[43] Eliciting cytosolic Ca^{2+} transients by voltage-clamping, we observed that during β-adrenergic stimulation (a situation when the heart requires increased ATP production), mitochondria take up Ca^{2+} rapidly, with $[Ca^{2+}]_m$ transients peaking slightly earlier than cytosolic Ca^{2+} transients.[43,44] This slightly earlier

$[Ca^{2+}]_m$ peak could not be explained by different dye kinetics and would be in agreement with the idea that mitochondria are located close to the SR where they would sense the RyR-released Ca^{2+} earlier (and at higher concentrations) than Ca^{2+} that is monitored by indo-1 in the bulk cytosol. Furthermore, $[Ca^{2+}]_m$ transients had 2.5-fold slower decay kinetics than $[Ca^{2+}]_c$.[43,44] This leads to accumulation of diastolic $[Ca^{2+}]_m$ during an increase in the amplitude and/or frequency of cytosolic Ca^{2+} transients. Blocking mitochondrial Ca^{2+} uptake with Ru360 reduced the mitochondrial Ca^{2+} transients by ~66%, whereas cytosolic Ca^{2+} transients slightly increased.[43,44] Blocking the primary mitochondrial Ca^{2+} export mechanism (i.e. the NCLX) increased diastolic $[Ca^{2+}]_m$ and decreased $[Ca^{2+}]_c$ transients,[43] while elevating $[Na^+]_i$ to activate the NCLX had the opposite effect, accelerating mitochondrial Ca^{2+} efflux, reducing steady-state $[Ca^{2+}]_m$ while slightly elevating $[Ca^{2+}]_c$.[43]

Since in our studies, rhod-2 was not calibrated to quantify $[Ca^{2+}]_m$, it remained unclear so far how much Ca^{2+} indeed enters mitochondria during a cytosolic Ca^{2+} transient. This question had spurred some controversy, since from indirect estimations (see below under 'Evidence for a mitochondrial microdomain') it seemed that mitochondria took up more Ca^{2+} than just 1% of overall cytosolic Ca^{2+} as originally proposed by the Bers group.[96,97] Maack *et al.*[43] and others[85,98] observed that when blocking mitochondrial Ca^{2+} uptake, the amplitude of cytosolic Ca^{2+} transients increased, although in our more recent studies,[44] this effect was smaller than in our initial study and no longer significant.[43]

Recently, the Bers group further addressed this issue. After their previous study using rhod-2 in permeabilized rat cardiac myocytes, in which they estimated that 2–10 nM Ca^{2+} enter mitochondria per beat,[75] they now used the mitochondrially targeted inverse pericam indicator Mitycam, which was previously established in cardiac myocytes by the group of Smith,[99] to measure mitochondrial Ca^{2+} uptake in intact rabbit cardiac myocytes.[76] With high spatial resolution microscopy, they observed that areas of mitochondria that are located within 500 nm from the dyads (i.e. the Z-line) have an amplitude of $[Ca^{2+}]_m$ of 37 nM per beat, while areas of mitochondria that are further (>500 nm) from the dyads have increases in $[Ca^{2+}]_m$ of 26 nM per cytosolic Ca^{2+} transient. Furthermore, the time-to-peak of $[Ca^{2+}]_m$ was shorter close to the Z-lines than in more remote areas of mitochondria, and since the MCU was evenly distributed over mitochondria, these data support the idea that spatial aspects (i.e. the existence of a mitochondrial Ca^{2+} microdomain; see also below) play an important role for the efficiency of mitochondrial Ca^{2+} uptake. Overall, these measurements support the concept that the quantities of mitochondrial Ca^{2+} uptake contribute only ~1% to cytosolic Ca^{2+} handling, which argues against the idea that mitochondrial Ca^{2+} uptake 'shapes' cytosolic Ca^{2+} transients in cardiac myocytes.

Although this study is presently the best quantitative estimation of mitochondrial Ca^{2+} uptake during EC coupling in cardiac myocytes, there are still some methodological issues that need to be taken into account when interpreting the data. To express Mitycam in cardiac myocytes, cells had to be cultured for 36 h before the functional experiments,[76] which may have an effect on the t-tubular organization of myocytes. Furthermore, the K_d of Mitycam for Ca^{2+} is ~200 nmol/L,[76,99] which may lead to saturation of fluorescence should $[Ca^{2+}]_m$ approach micromolar concentrations. A potentially important difference between this study[76] and our previous studies with rhod-2 AM[43,44,100] is that our experiments were carried out at 37°C in freshly isolated cardiac myocytes, and the times to peak (TTP) of cytosolic and mitochondrial Ca^{2+} transients were <60 ms, respectively[43,44,100]. In contrast, the study by Lu *et al.*[76] was carried out at room temperature, and TTP of $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ were between 200 and 300 ms. We observed that the rate of increase in $[Ca^{2+}]_c$ (which correlates with SR Ca^{2+} release flux)[101,102] had a strong impact on the efficiency of mitochondrial Ca^{2+} uptake. In conditions under which the TTP of $[Ca^{2+}]_c$ was 200–300 ms (i.e. in response to NCX-mediated Ca^{2+} influx triggering SR Ca^{2+} release), the efficiency of mitochondrial Ca^{2+} uptake was only 50% compared with conditions with rapid $I_{Ca,L}$-induced SR Ca^{2+} release.[100] Taken together, the study of Lu *et al.*[76] makes a strong and conclusive point that overall mitochondrial Ca^{2+} fluxes are rather low, but due to the mentioned technical limitations the actual mitochondrial Ca^{2+} uptake under 'real life' conditions may still be slightly underestimated. Nevertheless, the concept is further supported by a recent study that observed that the current of the MCU is dramatically lower in cardiac compared with skeletal muscle mitochondria of the mouse.[80]

Another level of complexity to the quantitative aspects of mitochondrial Ca^{2+} uptake and free matrix concentrations is added by the largely unknown Ca^{2+} buffering capacity of mitochondria. By measuring $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ in isolated mitochondria, Wei *et al.*[84] identified two modes of mitochondrial Ca^{2+} uptake, termed MCU_{mode1} and MCU_{mode2}. MCU_{mode1} mediated a large and rapid change of free $[Ca^{2+}]_m$ in response to small additions of extramitochondrial Ca^{2+}. In contrast, MCU_{mode2} mediated a slow and lower affinity Ca^{2+} uptake which was capable of taking up large amounts of Ca^{2+} that led to only relatively small changes of free $[Ca^{2+}]_m$. The different modes can be explained by access of these two MCU modes to different Ca^{2+} buffer systems, with MCU_{mode1} allowing pronounced changes in free $[Ca^{2+}]_m$ without major intramitochondrial Ca^{2+} buffering, potentially controlling Krebs cycle dehydrogenase activation for energy supply-and-demand matching.[84] In contrast, MCU_{mode2} has access to a dynamic phosphate-dependent buffer system that serves as a Ca^{2+} sink in pathological situations of cytosolic Ca^{2+} overload (e.g. during ischaemia/reperfusion).[84] In this respect, it was an interesting observation that the activation of the mPTP was uncorrelated with free $[Ca^{2+}]_m$ and may rather be mediated by a 'downstream by-product of $[Ca^{2+}]_m$, perhaps a Ca^{2+}-phosphate species'.[84]

5. Evidence for a mitochondrial Ca^{2+} microdomain

An important question is how mitochondria can take up Ca^{2+} at rapid kinetics considering that the $K_{0.5}$ of the MCU for Ca^{2+} is in the upper micromolar range, while the bulk cytosolic Ca^{2+} concentrations during systole are 1–2 orders of magnitude lower even during β-adrenergic stimulation. This seeming paradox would be resolved by the concept of a mitochondrial Ca^{2+} microdomain, in which mitochondria are in close vicinity to the RyRs of the SR (*Figure 1* and inset), a concept that is already well accepted in non-cardiac cells with regard to the interaction between the endoplasmic reticulum (ER) and mitochondria.[8] In fact, early electron micrographic studies revealed that in cardiac myocytes, the average distance between RyR2s and the mitochondrial surface ranges between 37 and 270 nm,[103] and in our more recent studies analysing transmission electron micrographs, the distance between the junctional SR and mitochondria averaged

15–20 nm.[68] Computational modelling predicts that the $[Ca^{2+}]$ at this distance from RyRs is at least 30 μmol/L during Ca^{2+} release.[11] When simulating such 'microdomain Ca^{2+} transients' by exposing mitochondria to brief Ca^{2+} pulses of 10–30 μmol/L in a computer model that integrates known kinetics of mitochondrial Ca^{2+} uptake and release with Krebs cycle metabolism, our experimental findings in cardiac myocytes could be reconciled.[43,45] Also functional data support the existence of a mitochondrial Ca^{2+} microdomain in cardiac myocytes: in cardiac H9c2 cells, mitochondrial Ca^{2+} uptake in response to caffeine-induced rapid SR Ca^{2+} release was comparable with exposing mitochondria to an extramitochondrial $[Ca^{2+}]$ of 30 μmol/L.[104] When adding Ca^{2+} buffers (EGTA or BAPTA) to the cytosol, the cytosolic, but not the mitochondrial Ca^{2+} transient could be suppressed after rapid caffeine-induced SR Ca^{2+} release in cardiac cells, indicating privileged Ca^{2+} communication between both organelles.[103,104] In mitochondria that were isolated by standard procedures from adult hearts, the application of caffeine elicited a Ca^{2+} transient in the mitochondrial matrix, but not in the extramitochondrial solution, which could be explained by Ca^{2+} transfer from the SR still being intimately linked to the OMM.[105] Two recent studies that used Ca^{2+}-sensitive fluorescent proteins genetically targeted to the OMM in non-cardiac cells or cardiac myotubes identified Ca^{2+} concentrations at the interface between the ER and mitochondria 5–10 times higher than in the bulk cytosol,[106,107] further supporting the idea that mitochondria are exposed to Ca^{2+} concentrations sufficient to overcome the low Ca^{2+} affinity of the MCU.

Recently, we tested the existence and physiological implications of a mitochondrial Ca^{2+} microdomain in adult guinea-pig cardiac myocytes by comparing the efficiency of mitochondrial Ca^{2+} uptake (indexed by the ratio of $[Ca^{2+}]_m$ over $[Ca^{2+}]_c$ increases in the same cells, respectively) after either a coordinated SR Ca^{2+} release or a trans-sarcolemmal Ca^{2+} influx via the reverse-mode of the NCX with Ca^{2+}-depleted SR.[100] The efficiency of mitochondrial Ca^{2+} uptake was twice as high after SR Ca^{2+} release compared with NCX-mediated Ca^{2+} influx, with a tight correlation between the rate of rise of the cytosolic Ca^{2+} transient and the efficiency of mitochondrial Ca^{2+} uptake.[100] This was of bioenergetic consequence, since the ratio of NAD(P)H/FAD, a sensitive index of Krebs cycle dehydrogenase activity, was increased more efficiently after SR Ca^{2+} release than after NCX-driven Ca^{2+} influx of similar magnitude. Taken together, these data strongly support the concept of privileged Ca^{2+} communication between mitochondria and the SR over other sources for cytosolic Ca^{2+} influx.

A very recent study by the Rizzuto group[85] used genetically encoded fluorescent Ca^{2+} indicators targeted to the cytosol, the OMM or the mitochondrial matrix in combination with genetic silencing or up-regulation of the MCU in neonatal cardiac myocytes and observed that (i) Ca^{2+} concentrations at the OMM clearly exceeded bulk cytosolic Ca^{2+} concentrations by several orders of magnitude, (ii) down-regulation of the MCU decreased and up-regulation of the MCU increased rapid $[Ca^{2+}]_m$ transients while (iii) the opposite occurred in the cytosol, where MCU down-regulation increased the amplitude of $[Ca^{2+}]_c$ transients and up-regulation of MCU decreased $[Ca^{2+}]_c$ transients. These data and some previous results[43] lend further support to the concept of a mitochondrial Ca^{2+} microdomain and—in contrast to other studies[75,76,108]—suggest that in cardiac myocytes, rapid mitochondrial Ca^{2+} uptake shapes cytosolic Ca^{2+} transients.

In this context, it is interesting to note that in atrial cardiac myocytes, the lack (or paucity) of T-tubules limits cytosolic Ca^{2+} transients to the subsarcolemmal space. Mackenzie *et al.*[109] observed that the propagation of subsarcolemmal Ca^{2+} transients to the central regions of the myocytes was limited by the SR and mitochondria, since inhibition of either SERCA or mitochondrial Ca^{2+} uptake increased the propagation of cytosolic Ca^{2+} to the central regions of the cell. These data support the idea that mitochondria could serve as a spatial buffer and thus, affect the magnitude and propagation of cytosolic Ca^{2+} transients.

6. Proteins that tether mitochondria to the SR

6.1 Role of mitofusin

Electron tomography studies on adult rat cardiac myocytes identified linker proteins that directly attach mitochondria to the SR, but whose molecular identity remained unresolved.[105,110] In a seminal study on mouse embryonic fibroblasts, de Brito and Scorrano[67] identified mitofusin (Mfn) 2 to physically link the ER to mitochondria. Mfns are proteins in the OMM with a well-characterized role in mitochondrial fusion (for a recent review, see Dorn and Maack[111]). Mfn1 and Mfn2 fuse mitochondria with each other by forming homo- and heterotypic complexes and thus, serve redundant roles for this process, so that deletion of *neither* Mfn1 *nor* Mfn2 alone affected mitochondrial fusion *in vivo*.[68,112] Since Mfn2 is also located to the cardiac SR,[113] it might serve as a linker between the SR and mitochondria, as revealed by de Brito and Scorrano[67] for ER-mitochondrial tethering in non-cardiac cells. To test this, we analysed mice with cardiomyocyte-specific post-natal deletion of either Mfn1 or Mfn2.[68] Up to an age of 6 weeks, these mice developed normal with no cardiac phenotype. However, transmission electron microscopy revealed that in Mfn2-, but not Mfn1-deficient mice, the contact length between the junctional SR and mitochondria was decreased, with a trend towards a widening of the gap between the two organelles. Likewise, RyR-containing mitochondrial associated membranes as a biochemical measure of SR-mitochondrial contact were strikingly decreased in Mfn2-KO mice.[68]

These data contrast somewhat with data from the Walsh group by Papanicolaou *et al.*,[114] who reported that cardiac-specific ablation of Mfn2 in mice did not alter the close associations between SR and mitochondria. There were, however, important methodological differences between our studies and those of Papanicolaou *et al.*.[114] First, we used a mouse model with post-natal knock-out of Mfn2,[68] while in the other study,[114] Mfn2 was knocked out already in the embryo, potentially facilitating mechanisms to compensate for the loss of Mfn2. Furthermore, Papanicolau *et al.*[114] analysed only the distance between mitochondria and the centre of T-tubules, which was in the range of 150 nm and not different between Mfn2-KO and control animals.[114] In contrast, we analysed the distance between the junctional SR and mitochondria, which is in the range of only 15 nm and a more direct parameter for SR-mitochondrial tethering.

To elucidate the functional relevance of these ultrastructural alterations, we performed experiments on isolated cardiac myocytes which were exposed to a physiological increase in workload (increased pacing frequency and β-adrenergic stimulation for 3 min). While in Mfn1 KO myocytes, mitochondrial Ca^{2+} uptake and the redox states of NAD(P)H and FAD were unchanged compared with WT littermates, in Mfn2-KO myocytes, mitochondrial Ca^{2+} uptake was

hampered during β-adrenergic stimulation at low stimulation frequencies.[68] At the same time, a transient oxidation of NAD(P)H and $FADH_2$ occurred, indicating that reduced mitochondrial Ca^{2+} uptake was associated with decreased Krebs cycle dehydrogenase activation. In agreement with our previous observation that the Krebs cycle also controls NADPH and ROS elimination,[43] a transient increase in ROS formation was observed in Mfn2-KO myocytes. Taken together, these results uncover critical roles for cardiomyocyte Mfn2 in tethering mitochondria to the junctional SR and creating a mitochondrial Ca^{2+} microdomain. Disruption of SR-mitochondrial cross-talk hampers energy supply-and-demand matching, and possibly provokes oxidative stress. At the same time, these data are in line with previous reports[43,85,98] that mitochondrial Ca^{2+} uptake shapes cytosolic Ca^{2+} transients.

6.2 Other tethering proteins

Previous studies in non-cardiac cells have revealed several proteins involved in tethering the ER to mitochondria. Already in 2002, Rizzuto's group identified voltage-dependent anion channel (VDAC), a well-characterized Ca^{2+} permitting protein in the OMM to govern Ca^{2+} transmission from the ER to mitochondria in HeLa cells.[115] Subsequently, the same group found that VDAC is physically linked to the ER Ca^{2+} release channel, the inositol 1,4,5-triphosphate receptor (IP_3R) by glucose-regulated protein 75 (grp75).[116] In a more recent study, Hajnoczky's group observed that during cardiomyocyte development, the ER-mitochondrial Ca^{2+} transfer that is governed by IP_3Rs in early developmental stages of cardiomyocytes transforms to a RyR2-dependent SR-mitochondrial Ca^{2+} transfer.[117] In agreement with this concept, a recent biochemical study revealed physical interaction between RyR2 and VDAC2 with co-localization in SR-mitochondrial junctions in HL-1 cells (*Figure 1*).[118] It is currently unclear whether this interaction is direct or maybe mediated by another tethering protein-like grp75 (which tethers IP_3Rs to VDAC).[116] Other studies in non-cardiac cells identified the Mmm1/Mdm10/Mdm12/Mdm34 complex[119] and PACS-2[120] as linker proteins between the ER and mitochondria. Their role in cardiac myocytes, however, is currently unknown.

The role of tethering proteins is obviously thought to create Ca^{2+} microdomains between the SR and the OMM, and recent evidence suggests that the areas of the OMM which are tethered closely to the SR are also closer to the IMM than areas of the OMM that do not have a close SR contact[113] (*Figure 1*, inset). In fact, these contact points between IMM and OMM are enriched in VDAC,[113] which associates with RyR2,[118] and a lighter version of the Mfn2 protein (50 kDa), while those areas of the OMM that are not engaged in contact points harbour the full-length Mfn2 protein (~80 kDa).[113] While the functional implications of the lighter Mfn2 protein are presently unclear, these data nevertheless suggest that the contact points function as anchorage sites for the SR-mitochondrial physical coupling, and that close coupling of the SR, OMM, and IMM is likely to 'provide a favourable spatial arrangement for local RyR2-mitochondrial Ca^{2+} signalling'.[113]

7. Pathophysiological changes in heart failure

In chronic heart failure, maladaptive remodelling of EC coupling induces contractile dysfunction. The most prominent changes are (i) decreased SR Ca^{2+} load, (ii) an elevation of $[Na^+]_i$ that reduces cytosolic Ca^{2+} efflux (via the *forward* mode of the NCX) and increases Ca^{2+} influx (via the *reverse* mode; *Figure 1*), and (iii) t-tubular remodelling that induces a dyssynchronization of cytosolic Ca^{2+} transients.[5,12–16] Irrespective of the absolute quantities of mitochondrial Ca^{2+} uptake during EC coupling, all these processes have a negative impact on mitochondrial Ca^{2+} uptake. First, the amplitudes of mitochondrial Ca^{2+} transients correlate with the amplitudes of cytosolic Ca^{2+} transients,[43,44,100] which are decreased in failing cardiac myocytes.[25] Furthermore, we observed that reverse-mode NCX-driven Ca^{2+} influx is less efficient for mitochondrial Ca^{2+} uptake than coordinated SR Ca^{2+} release (due to its slower kinetics and less privileged localization to mitochondria; *Figure 1*).[100] Similarly, t-tubular dysorganization leads to dyssynchronized and thus, slower cytosolic Ca^{2+} transients, which based on our experimental results[100] should also negatively affect mitochondrial Ca^{2+} uptake. A study on human cardiac mitoplasts revealed that the opening probability of the mCa1 (equalling MCU) and mCa2 were reduced in the mitochondria from patients with heart failure.[83] Finally, since Ca^{2+} is exported from the mitochondria by the NCLX, the elevation of $[Na^+]_i$ observed in failing myocytes reduces steady-state $[Ca^{2+}]_m$ and leads to an oxidation of NADH and NADPH, which on the one hand impairs energy supply-and-demand matching and on the other hand leads to oxidative stress[43,44,121] (*Figure 1*). Increased ROS production, in turn, activates CaMKII,[122] which elevates the late I_{Na}[30] and thus, $[Na^+]_i$, inducing a positive feedback-loop that sustains defects in EC coupling, energetics deficit and oxidative stress.[35] Furthermore, mitochondrial ROS activate RyRs and thus, have a direct feedback on SR Ca^{2+} release events, which may further contribute to SR Ca^{2+} leak and arrhythmias (*Figure 1*).[60–62]

8. Conclusions

Considerable progress has been made on identifying the mechanisms and molecular determinants of mitochondrial Ca^{2+} uptake and release in cardiac myocytes. In particular, the identification of the MCU, its regulatory factors MICU1 and MCUR1 as well as of the NCLX, but also tethering proteins between the SR and mitochondria, such as Mfn2, RyR2, and VDAC will allow further characterization of the processes of mitochondrial Ca^{2+} uptake and release in cardiac myocytes. These studies together with the further improvement of mitochondrially located Ca^{2+} indicators will hopefully resolve some of the remaining open issues such as the exact quantities and kinetics of mitochondrial Ca^{2+} uptake during EC coupling and its role for cytosolic Ca^{2+}.

Conflict of interest: none declared.

Funding

The authors are supported by the Deutsche Forschungsgemeinschaft (DFG; Heisenberg Programm, SFB 894 and Klinische Forschergruppe 196).

References

1. Harris DA, Das AM. Control of mitochondrial ATP synthesis in the heart. *Biochem J* 1991;**280**(Pt 3):561–573.
2. Mootha VK, Arai AE, Balaban RS. Maximum oxidative phosphorylation capacity of the mammalian heart. *Am J Physiol* 1997;**272**:H769–H775.
3. Balaban RS. Cardiac energy metabolism homeostasis: role of cytosolic calcium. *J Mol Cell Cardiol* 2002;**34**:1259–1271.
4. Brandes R, Bers DM. Intracellular Ca^{2+} increases the mitochondrial NADH concentration during elevated work in intact cardiac muscle. *Circ Res* 1997;**80**:82–87.

5. Maack C, O'Rourke B. Excitation-contraction coupling and mitochondrial energetics. *Basic Res Cardiol* 2007;**102**:369–392.
6. Balaban RS. Domestication of the cardiac mitochondrion for energy conversion. *J Mol Cell Cardiol* 2009;**46**:832–841.
7. Cortassa S, Aon MA, O'Rourke B, Jacques R, Tseng HJ, Marban Eet *al*. A computational model integrating electrophysiology, contraction, and mitochondrial bioenergetics in the ventricular myocyte. *Biophys J* 2006;**91**:1564–1589.
8. Rizzuto R, Pozzan T. Microdomains of intracellular Ca^{2+}: molecular determinants and functional consequences. *Physiol Rev* 2006;**86**:369–408.
9. Saks V, Dzeja P, Schlattner U, Vendelin M, Terzic A, Wallimann T. Cardiac system bioenergetics: metabolic basis of the Frank-Starling law. *J Physiol* 2006;**571**:253–273.
10. Bers DM. Calcium cycling and signaling in cardiac myocytes. *Annu Rev Physiol* 2008;**70**:23–49.
11. Peskoff A, Langer GA. Calcium concentration and movement in the ventricular cardiac cell during an excitation-contraction cycle. *Biophys J* 1998;**74**:153–174.
12. Bers DM. Altered cardiac myocyte Ca regulation in heart failure. *Physiology (Bethesda)* 2006;**21**:380–387.
13. Houser SR, Margulies KB. Is depressed myocyte contractility centrally involved in heart failure?. *Circ Res* 2003;**92**:350–358.
14. Heinzel FR, MacQuaide N, Biesmans L, Sipido K. Dyssynchrony of Ca^{2+} release from the sarcoplasmic reticulum as subcellular mechanism of cardiac contractile dysfunction. *J Mol Cell Cardiol* 2011;**50**:390–400.
15. Bito V, Heinzel FR, Biesmans L, Antoons G, Sipido KR. Crosstalk between L-type Ca^{2+} channels and the sarcoplasmic reticulum: alterations during cardiac remodelling. *Cardiovasc Res* 2008;**77**:315–324.
16. Lyon AR, MacLeod KT, Zhang Y, Garcia E, Kanda GK, Lab MJet *al*. Loss of T-tubules and other changes to surface topography in ventricular myocytes from failing human and rat heart. *Proc Natl Acad Sci USA* 2009;**106**:6854–6859.
17. Despa S, Islam MA, Weber CR, Pogwizd SM, Bers DM. Intracellular Na(+) concentration is elevated in heart failure but Na/K pump function is unchanged. *Circulation* 2002;**105**:2543–2548.
18. Pieske B, Houser SR. [Na+]i handling in the failing human heart. *Cardiovasc Res* 2003;**57**:874–886.
19. Pieske B, Maier LS, Piacentino V III, Weisser J, Hasenfuss G, Houser S. Rate dependence of $[Na^+]_i$ and contractility in nonfailing and failing human myocardium. *Circulation* 2002;**106**:447–453.
20. Baartscheer A, Schumacher CA, van Borren MM, Belterman CN, Coronel R, Fiolet JW. Increased Na+/H+-exchange activity is the cause of increased $[Na^+]_i$ and underlies disturbed calcium handling in the rabbit pressure and volume overload heart failure model. *Cardiovasc Res* 2003;**57**:1015–1024.
21. O'Rourke B, Maack C. The role of Na dysregulation in cardiac disease and how it impacts electrophysiology. *Drug Discov Today Dis Models* 2007;**4**:207–217.
22. Baartscheer A, Schumacher CA, Belterman CN, Coronel R, Fiolet JW. $[Na^+]_i$ and the driving force of the Na^+/Ca^{2+}-exchanger in heart failure. *Cardiovasc Res* 2003;**57**:986–995.
23. Armoundas AA, Hobai IA, Tomaselli GF, Winslow RL, O'Rourke B. Role of sodium-calcium exchanger in modulating the action potential of ventricular myocytes from normal and failing hearts. *Circ Res* 2003;**93**:46–53.
24. Weber CR, Piacentino V III, Houser SR, Bers DM. Dynamic regulation of sodium/calcium exchange function in human heart failure. *Circulation* 2003;**108**:2224–2229.
25. Piacentino V III, Weber CR, Chen X, Weisser-Thomas J, Margulies KB, Bers DMet *al*. Cellular basis of abnormal calcium transients of failing human ventricular myocytes. *Circ Res* 2003;**92**:651–658.
26. Weisser-Thomas J, Piacentino V III, Gaughan JP, Margulies K, Houser SR. Calcium entry via Na/Ca exchange during the action potential directly contributes to contraction of failing human ventricular myocytes. *Cardiovasc Res* 2003;**57**:974–985.
27. Dipla K, Mattiello JA, Margulies KB, Jeevanandam V, Houser SR. The sarcoplasmic reticulum and the $Na+/Ca^{2+}$ exchanger both contribute to the Ca^{2+} transient of failing human ventricular myocytes. *Circ Res* 1999;**84**:435–444.
28. Grimm M, Brown JH. Beta-adrenergic receptor signaling in the heart: role of CaMKII. *J Mol Cell Cardiol* 2010;**48**:322–330.
29. Maier LS, Bers DM. Role of Ca^{2+}/calmodulin-dependent protein kinase (CaMK) in excitation-contraction coupling in the heart. *Cardiovasc Res* 2007;**73**:631–640.
30. Wagner S, Dybkova N, Rasenack EC, Jacobshagen C, Fabritz L, Kirchhof Pet *al*. Ca^{2+}/calmodulin-dependent protein kinase II regulates cardiac Na^+ channels. *J Clin Invest* 2006;**116**:3127–3138.
31. Ingwall JS, Weiss RG. Is the failing heart energy starved? On using chemical energy to support cardiac function. *Circ Res* 2004;**95**:135–145.
32. Weiss RG, Gerstenblith G, Bottomley PA. ATP flux through creatine kinase in the normal, stressed, and failing human heart. *Proc Natl Acad Sci USA* 2005;**102**:808–813.
33. Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. *J Clin Invest* 2005;**115**:500–508.
34. Li Y, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JLet *al*. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* 1995;**11**:376–381.
35. Kohlhaas M, Maack C. Interplay of defective excitation-contraction coupling, energy starvation, and oxidative stress in heart failure. *Trends Cardiovasc Med* 2011;**21**:69–73.
36. O-Uchi J, Pan S, Sheu SS. Perspectives on: SGP symposium on mitochondrial physiology and medicine: molecular identities of mitochondrial Ca^{2+} influx mechanism: updated passwords for accessing mitochondrial Ca^{2+}-linked health and disease. *J Gen Physiol* 2012;**139**:435–443.
37. Dedkova EN, Blatter LA. Calcium signaling in cardiac mitochondria. *J Mol Cell Cardiol* 2013. Advance Access published January 7, 2013, doi:10.1016/j.yjmcc.2012.12.021.
38. Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Yet *al*. Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. *Nature* 2011;**476**:341–345.
39. De Stefani D, Raffaello A, Teardo E, Szabo I, Rizzuto R. A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. *Nature* 2011;**476**:336–340.
40. Brandes R, Bers DM. Increased work in cardiac trabeculae causes decreased mitochondrial NADH fluorescence followed by slow recovery. *Biophys J* 1996;**71**:1024–1035.
41. Brandes R, Bers DM. Analysis of the mechanisms of mitochondrial NADH regulation in cardiac trabeculae. *Biophys J* 1999;**77**:1666–1682.
42. Brandes R, Bers DM. Simultaneous measurements of mitochondrial NADH and Ca(2+) during increased work in intact rat heart trabeculae. *Biophys J* 2002;**83**:587–604.
43. Maack C, Cortassa S, Aon MA, Ganesan AN, Liu T, O'Rourke B. Elevated cytosolic Na+ decreases mitochondrial Ca^{2+} uptake during excitation-contraction coupling and impairs energetic adaptation in cardiac myocytes. *Circ Res* 2006;**99**:172–182.
44. Kohlhaas M, Liu T, Knopp A, Zeller T, Ong MF, Böhm Met *al*. Elevated cytosolic Na^+ increases mitochondrial formation of reactive oxygen species in failing cardiac myocytes. *Circulation* 2010;**121**:1606–1613.
45. Cortassa S, Aon MA, Marban E, Winslow RL, O'Rourke B. An integrated model of cardiac mitochondrial energy metabolism and calcium dynamics. *Biophys J* 2003;**84**:2734–2755.
46. Cortassa S, O'Rourke B, Winslow RL, Aon MA. Control and regulation of mitochondrial energetics in an integrated model of cardiomyocyte function. *Biophys J* 2009;**96**:2466–2478.
47. Glancy B, Balaban RS. Role of mitochondrial Ca^{2+} in the regulation of cellular energetics. *Biochemistry* 2012;**51**:2959–2973.
48. Balaban RS, Kantor HL, Katz LA, Briggs RW. Relation between work and phosphate metabolite in the *in vivo* paced mammalian heart. *Science* 1986;**232**:1121–1123.
49. Katz LA, Swain JA, Portman MA, Balaban RS. Relation between phosphate metabolites and oxygen consumption of heart *in vivo*. *Am J Physiol* 1989;**256**:H265–H274.
50. Katz LA, Koretsky AP, Balaban RS. Respiratory control in the glucose perfused heart. A 31P NMR and NADH fluorescence study. *FEBS Lett* 1987;**221**:270–276.
51. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. *Cell* 2005;**120**:483–495.
52. Aon MA, Cortassa S, Marban E, O'Rourke B. Synchronized whole cell oscillations in mitochondrial metabolism triggered by a local release of reactive oxygen species in cardiac myocytes. *J Biol Chem* 2003;**278**:44735–44744.
53. Aon MA, Cortassa S, O'Rourke B. Redox-optimized ROS balance: a unifying hypothesis. *Biochim Biophys Acta* 2010;**1797**:865–877.
54. Dedkova EN, Blatter LA. Characteristics and function of cardiac mitochondrial nitric oxide synthase. *J Physiol* 2009;**587**:851–872.
55. Brookes PS. Mitochondrial nitric oxide synthase. *Mitochondrion* 2004;**3**:187–204.
56. Kaludercic N, Takimoto E, Nagayama T, Feng N, Lai EW, Bedja Det *al*. Monoamine oxidase A-mediated enhanced catabolism of norepinephrine contributes to adverse remodeling and pump failure in hearts with pressure overload. *Circ Res* 2010;**106**:193–202.
57. Kuroda J, Ago T, Matsushima S, Zhai P, Schneider MD, Sadoshima J. NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. *Proc Natl Acad Sci USA* 2010;**107**:15565–15570.
58. Ago T, Kuroda J, Pain J, Fu C, Li H, Sadoshima J. Upregulation of Nox4 by hypertrophic stimuli promotes apoptosis and mitochondrial dysfunction in cardiac myocytes. *Circ Res* 2010;**106**:1253–1264.
59. Ying W. NAD+/NADH and NADP+/NADPH in cellular functions and cell death: regulation and biological consequences. *Antioxid Redox Signal* 2008;**10**:179–206.
60. Zima AV, Blatter LA. Redox regulation of cardiac calcium channels and transporters. *Cardiovasc Res* 2006;**71**:310–321.
61. Zhou L, Aon MA, Liu T, O'Rourke B. Dynamic modulation of Ca^{2+} sparks by mitochondrial oscillations in isolated guinea pig cardiomyocytes under oxidative stress. *J Mol Cell Cardiol* 2011;**51**:632–639.
62. Yan Y, Liu J, Wei C, Li K, Xie W, Wang Yet *al*. Bidirectional regulation of Ca^{2+} sparks by mitochondria-derived reactive oxygen species in cardiac myocytes. *Cardiovasc Res* 2008;**77**:432–441.
63. Prosser BL, Ward CW, Lederer WJ. X-ROS signaling: rapid mechano-chemo transduction in heart. *Science* 2011;**333**:1440–1445.
64. Palty R, Silverman WF, Hershfinkel M, Caporale T, Sensi SL, Parnis Jet *al*. NCLX is an essential component of mitochondrial $Na+/Ca^{2+}$ exchange. *Proc Natl Acad Sci USA* 2010;**107**:436–441.
65. Mallilankaraman K, Doonan P, Cardenas C, Chandramoorthy HC, Muller M, Miller Ret *al*. MICU1 is an essential gatekeeper for MCU-mediated mitochondrial Ca(2+) uptake that regulates cell survival. *Cell* 2012;**151**:630–644.

66. Perocchi F, Gohil VM, Girgis HS, Bao XR, McCombs JE, Palmer AE*et al.* MICU1 encodes a mitochondrial EF hand protein required for Ca(2+) uptake. *Nature* 2010;**467**:291–296.
67. de Brito OM, Scorrano L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* 2008;**456**:605–610.
68. Chen Y, Csordas G, Jowdy C, Schneider TG, Csordas N, Wang W*et al.* Mitofusin 2-containing mitochondrial-reticular microdomains direct rapid cardiomyocyte bioenergetic responses via interorganelle Ca^{2+} crosstalk. *Circ Res* 2012;**111**:863–875.
69. Mallilankaraman K, Cardenas C, Doonan PJ, Chandramoorthy HC, Irrinki KM, Golenar T*et al.* MCUR1 is an essential component of mitochondrial Ca^{2+} uptake that regulates cellular metabolism. *Nat Cell Biol* 2012;**14**:1336–1343.
70. O'Rourke B, Blatter LA. Mitochondrial Ca^{2+} uptake: tortoise or hare? *J Mol Cell Cardiol* 2009;**46**:767–774.
71. Dedkova EN, Blatter LA. Mitochondrial Ca^{2+} and the heart. *Cell Calcium* 2008;**44**: 77–91.
72. Chikando AC, Kettlewell S, Williams GS, Smith G, Lederer WJ. Ca^{2+} dynamics in the mitochondria—state of the art. *J Mol Cell Cardiol* 2011;**51**:627–631.
73. Hüser J, Blatter LA, Sheu SS. Mitochondrial calcium in heart cells: beat-to-beat oscillations or slow integration of cytosolic transients? *J Bioenerg Biomembr* 2000;**32**: 27–33.
74. Dedkova EN, Blatter LA. Measuring mitochondrial function in intact cardiac myocytes. *J Mol Cell Cardiol* 2012;**52**:48–61.
75. Andrienko TN, Picht E, Bers DM. Mitochondrial free calcium regulation during sarcoplasmic reticulum calcium release in rat cardiac myocytes. *J Mol Cell Cardiol* 2009;**46**:1027–1036.
76. Lu X, Ginsburg KS, Kettlewell S, Bossuyt J, Smith GL, Bers DM. Measuring local gradients of intramitochondrial $[Ca^{2+}]$ in cardiac myocytes during sarcoplasmic reticulum Ca^{2+} release. *Circ Res* 2013;**112**:424–431.
77. Pacher P, Csordas P, Schneider T, Hajnoczky G. Quantification of calcium signal transmission from sarco-endoplasmic reticulum to the mitochondria. *J Physiol* 2000;**529**(Pt 3):553–564.
78. Kirichok Y, Krapivinsky G, Clapham DE. The mitochondrial calcium uniporter is a highly selective ion channel. *Nature* 2004;**427**:360–364.
79. Hille B. *Ion Channels of Excitable Membranes*. Sunderland, MA: Sinauer Associates 2001.
80. Fieni F, Bae Lee S, Jan YN, Kirichok Y. Activity of the mitochondrial calcium uniporter varies greatly between tissues. *Nat Commun* 2012;**3**:1317.
81. Gunter TE, Pfeiffer DR. Mechanisms by which mitochondria transport calcium. *Am J Physiol* 1990;**258**:C755–C786.
82. Gunter TE, Gunter KK, Sheu SS, Gavin CE. Mitochondrial calcium transport: physiological and pathological relevance. *Am J Physiol* 1994;**267**:C313–339.
83. Michels G, Khan IF, Endres-Becker J, Rottlaender D, Herzig S, Ruhparwar A*et al.* Regulation of the human cardiac mitochondrial Ca^{2+} uptake by 2 different voltage-gated Ca^{2+} channels. *Circulation* 2009;**119**:2435–2443.
84. Wei AC, Liu T, Winslow RL, O'Rourke B. Dynamics of matrix-free Ca^{2+} in cardiac mitochondria: two components of Ca^{2+} uptake and role of phosphate buffering. *J Gen Physiol* 2012;**139**:465–478.
85. Drago I, De Stefani D, Rizzuto R, Pozzan T. Mitochondrial Ca^{2+} uptake contributes to buffering cytoplasmic Ca^{2+} peaks in cardiomyocytes. *Proc Natl Acad Sci USA* 2012; **109**:12986–12991.
86. Miyata H, Silverman HS, Sollott SJ, Lakatta EG, Stern MD, Hansford RG. Measurement of mitochondrial free Ca^{2+} concentration in living single rat cardiac myocytes. *Am J Physiol* 1991;**261**:H1123–H1134.
87. Halestrap A. Biochemistry: a pore way to die. *Nature* 2005;**434**:578–579.
88. Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu SS. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. *Am J Physiol Cell Physiol* 2004;**287**:C817–C833.
89. Kung G, Konstantinidis K, Kitsis RN. Programmed necrosis, not apoptosis, in the heart. *Circ Res* 2011;**108**:1017–1036.
90. Joiner ML, Koval OM, Li J, He BJ, Allamargot C, Gao Z*et al.* CaMKII determines mitochondrial stress responses in heart. *Nature* 2012;**491**:269–273.
91. Denton RM, McCormack JG. Ca^{2+} transport by mammalian mitochondria and its role in hormone action. *Am J Physiol* 1985;**249**:E543–E554.
92. Baysal K, Jung DW, Gunter KK, Gunter TE, Brierley GP. Na(+)-dependent Ca^{2+} efflux mechanism of heart mitochondria is not a passive Ca^{2+}/2Na+ exchanger. *Am J Physiol* 1994;**266**:C800–C808.
93. Jung DW, Baysal K, Brierley GP. The sodium-calcium antiport of heart mitochondria is not electroneutral. *J Biol Chem* 1995;**270**:672–678.
94. Paucek P, Jaburek M. Kinetics and ion specificity of Na(+)/Ca(2+) exchange mediated by the reconstituted beef heart mitochondrial Na(+)/Ca(2+) antiporter. *Biochim Biophys Acta* 2004;**1659**:83–91.
95. Pogwizd SM, Sipido KR, Verdonck F, Bers DM. Intracellular Na in animal models of hypertrophy and heart failure: contractile function and arrhythmogenesis. *Cardiovasc Res* 2003;**57**:887–896.
96. Bassani RA, Bassani JW, Bers DM. Mitochondrial and sarcolemmal Ca^{2+} transport reduce $[Ca^{2+}]$i during caffeine contractures in rabbit cardiac myocytes. *J Physiol* 1992;**453**:591–608.
97. Zhou Z, Matlib MA, Bers DM. Cytosolic and mitochondrial Ca^{2+} signals in patch clamped mammalian ventricular myocytes. *J Physiol* 1998;**507**(Pt 2):379–403.
98. Isenberg G, Han S, Schiefer A, Wendt-Gallitelli MF. Changes in mitochondrial calcium concentration during the cardiac contraction cycle. *Cardiovasc Res* 1993;**27**:1800–1809.
99. Kettlewell S, Cabrero P, Nicklin SA, Dow JA, Davies S, Smith GL. Changes of intramitochondrial Ca^{2+} in adult ventricular cardiomyocytes examined using a novel fluorescent Ca^{2+} indicator targeted to mitochondria. *J Mol Cell Cardiol* 2009;**46**:891–901.
100. Kohlhaas M, Maack C. Adverse bioenergetic consequences of Na^{+}-Ca^{2+} exchanger-mediated Ca^{2+} influx in cardiac myocytes. *Circulation* 2010;**122**:2273–2280.
101. Sipido KR, Maes M, Van de Werf F. Low efficiency of Ca^{2+} entry through the Na(+)-Ca^{2+} exchanger as trigger for Ca^{2+} release from the sarcoplasmic reticulum. A comparison between L-type Ca^{2+} current and reverse-mode Na(+)-Ca^{2+} exchange. *Circ Res* 1997;**81**:1034–1044.
102. Sipido KR, Wier WG. Flux of Ca^{2+} across the sarcoplasmic reticulum of guinea-pig cardiac cells during excitation-contraction coupling. *J Physiol* 1991;**435**:605–630.
103. Sharma VK, Ramesh V, Franzini-Armstrong C, Sheu SS. Transport of Ca^{2+} from sarcoplasmic reticulum to mitochondria in rat ventricular myocytes. *J Bioenerg Biomembr* 2000;**32**:97–104.
104. Szalai G, Csordas G, Hantash BM, Thomas AP, Hajnoczky G. Calcium signal transmission between ryanodine receptors and mitochondria. *J Biol Chem* 2000;**275**:15305–15313.
105. Garcia-Perez C, Hajnoczky G, Csordas G. Physical coupling supports the local Ca^{2+} transfer between sarcoplasmic reticulum subdomains and the mitochondria in heart muscle. *J Biol Chem* 2008;**283**:32771–32780.
106. Csordas G, Varnai P, Golenar T, Roy S, Purkins G, Schneider TGet *al.* Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface. *Mol Cell* 2010;**39**:121–132.
107. Giacomello M, Drago I, Bortolozzi M, Scorzeto M, Gianelle A, Pizzo Pet *al.* Ca^{2+} hot spots on the mitochondrial surface are generated by Ca^{2+} mobilization from stores, but not by activation of store-operated Ca^{2+} channels. *Mol Cell* 2010;**38**:280–290.
108. Sedova M, Dedkova EN, Blatter LA. Integration of rapid cytosolic Ca^{2+} signals by mitochondria in cat ventricular myocytes. *Am J Physiol Cell Physiol* 2006;**291**: C840–850.
109. Mackenzie L, Roderick HL, Berridge MJ, Conway SJ, Bootman MD. The spatial pattern of atrial cardiomyocyte calcium signalling modulates contraction. *J Cell Sci* 2004;**117**:6327–6337.
110. Hayashi T, Martone ME, Yu Z, Thor A, Doi M, Holst MJ*et al.* Three-dimensional electron microscopy reveals new details of membrane systems for Ca^{2+} signaling in the heart. *J Cell Sci* 2009;**122**:1005–1013.
111. Dorn GW II, Maack C. SR and mitochondria: calcium cross-talk between kissing cousins. *J Mol Cell Cardiol* 2013;**55**:42–49.
112. Chen Y, Liu Y, Dorn GW II. Mitochondrial fusion is essential for organelle function and cardiac homeostasis. *Circ Res* 2011;**109**:1327–1331.
113. Garcia-Perez C, Schneider TG, Hajnoczky G, Csordas G. Alignment of sarcoplasmic reticulum-mitochondrial junctions with mitochondrial contact points. *Am J Physiol Heart Circ Physiol* 2011;**301**:H1907–H1915.
114. Papanicolaou KN, Khairallah RJ, Ngoh GA, Chikando A, Luptak I, O'Shea KM*et al.* Mitofusin-2 maintains mitochondrial structure and contributes to stress-induced permeability transition in cardiac myocytes. *Mol Cell Biol* 2011;**31**:1309–1328.
115. Rapizzi E, Pinton P, Szabadkai G, Wieckowski MR, Vandecasteele G, Baird G*et al.* Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca^{2+} microdomains to mitochondria. *J Cell Biol* 2002;**159**:613–624.
116. Szabadkai G, Bianchi K, Varnai P, De Stefani D, Wieckowski MR, Cavagna D*et al.* Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca^{2+} channels. *J Cell Biol* 2006;**175**:901–911.
117. Yi M, Weaver D, Eisner V, Varnai P, Hunyady L, Ma J*et al.* Switch from ER-mitochondrial to SR-mitochondrial calcium coupling during muscle differentiation. *Cell Calcium* 2012;**52**:355–365.
118. Min CK, Yeom DR, Lee KE, Kwon HK, Kang M, Kim YS*et al.* Coupling of ryanodine receptor 2 and voltage-dependent anion channel 2 is essential for Ca^{2+} transfer from the sarcoplasmic reticulum to the mitochondria in the heart. *Biochem J* 2012; **447**:371–379.
119. Kornmann B, Currie E, Collins SR, Schuldiner M, Nunnari J, Weissman JS*et al.* An ER-mitochondria tethering complex revealed by a synthetic biology screen. *Science* 2009;**325**:477–481.
120. Simmen T, Aslan JE, Blagoveshchenskaya AD, Thomas L, Wan L, Xiang Y*et al.* PACS-2 controls endoplasmic reticulum-mitochondria communication and Bid-mediated apoptosis. *EMBO J* 2005;**24**:717–729.
121. Liu T, O'Rourke B. Enhancing mitochondrial Ca^{2+} uptake in myocytes from failing hearts restores energy supply and demand matching. *Circ Res* 2008;**103**:279–288.
122. Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV*et al.* A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. *Cell* 2008;**133**:462–474.