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Review

Mitochondria in cardiomyocyte Ca^{2+} signaling

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ABSTRACT

Ca^{2+} signaling is of vital importance to cardiac cell function and plays an important role in heart failure. It is based on sarcolemmal, sarcoplasmic reticulum and mitochondrial Ca^{2+} cycling. While the first two are well characterized, the latter remains unclear, controversial and technically challenging.

In mammalian cardiac myocytes, Ca^{2+} influx through L-type calcium channels in the sarcolemmal membrane triggers Ca^{2+} release from the nearby junctional sarcoplasmic reticulum to produce Ca^{2+} sparks. When this triggering is synchronized by the cardiac action potential, a global $[Ca^{2+}]_i$ transient arises from coordinated Ca^{2+} release events. The ends of intermyofibrillar mitochondria are located within 20 nm of the junctional sarcoplasmic reticulum and thereby experience a high local $[Ca^{2+}]$ during the Ca^{2+} release process. Both local and global Ca^{2+} signals may thus influence calcium signaling in mitochondria and, reciprocally, mitochondria may contribute to the local control of calcium signaling. In addition to the intermyofibrillar mitochondria, morphologically distinct mitochondria are also located in the perinuclear and subsarcolemmal regions of the cardiomyocyte and thus experience a different local $[Ca^{2+}]$.

Here we review the literature in regard to several issues of broad interest: (1) the ultrastructural basis for mitochondrion – sarcoplasmic reticulum cross-signaling; (2) mechanisms of sarcoplasmic reticulum signaling; (3) mitochondrial calcium signaling; and (4) the possible interplay of calcium signaling between the sarcoplasmic reticulum and adjacent mitochondria.

Finally, this review discusses experimental findings and mathematical models of cardiac calcium signaling between the sarcoplasmic reticulum and mitochondria, identifies weaknesses in these models, and suggests strategies and approaches for future investigations.

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1. Introduction

The release of Ca^{2+} from the sarcoplasmic reticulum (SR) is central to the normal physiology of cardiac myocytes, and the cellular and subcellular control of intracellular calcium concentration ($[Ca^{2+}]_i$) is the focus of much current research. The major mechanism by which the cardiac action potential (AP) triggers Ca^{2+} release from the SR is by Ca^{2+}-induced Ca^{2+} release (Bers, 2001, 2002a,b; Cannell et al., 1995; Fabiato, 1985, 1992). The released Ca^{2+}, after having activated contractile elements, is pumped back into the SR by SR Ca^{2+}-ATPase (SERCA2a). The global $[Ca^{2+}]_i$ elevation is restored to the low diastolic $[Ca^{2+}]_i$ when Ca^{2+} is removed from the cytoplasm by the sarcolemmal Ca^{2+}-ATPase and the Na^+/Ca^{2+}-exchanger. Some of the elevated $[Ca^{2+}]_i$ may also be buffered by the mitochondria, with entry into the matrix permitted down an electrochemical gradient through a channel known as the mitochondrial Ca^{2+} uniporter (MCU). In the complete cycle of Ca^{2+} elevation and reduction, the Ca^{2+} levels in each compartment at the end of the cycle must be restored to the pre-release state. Thus the amount of Ca^{2+} released from the SR (triggered by I_{Ca}, the sarcolemmal voltage-dependent Ca^{2+} channel current) must be equal to the amount of Ca^{2+} taken up by the SR in the steady-state. This requirement for steady-state Ca^{2+} flux balance applies not only to SR Ca^{2+} cycling (Bers, 2001; Eisner et al., 2000), but also for uptake and release of Ca^{2+} across the sarcolemma and Ca^{2+} entry and exit for the mitochondria.

This cellular and subcellular Ca^{2+} cycling is of vital importance to cardiac cell function and plays an important role in ventricular dysfunctions such as heart failure. The regulation of this system *in vivo* is an area of active investigation by many laboratories. An important clue to understanding these regulatory mechanisms may come from the recognition that the control of the Ca^{2+} cycling, and therefore signal transduction, occurs in spatially discrete subdomains, as suggested earlier for Ca^{2+}-induced Ca^{2+} release (Izu and Balke, 2002; Niggli and Lederer, 1990; Santana et al., 1996; Stern, 1992; Stern et al., 1999; Wier et al., 1994). For example, when local-control mechanisms dominate, the triggering of SR Ca^{2+} release channels (type 2 ryanodine receptors, RyR2s) is governed not by the global, cell averaged $[Ca^{2+}]$, but instead by the Ca^{2+} microdomain surrounding each cluster of RyR2s at the junctional SR (jSR), due initially to the influx of Ca^{2+} from sarcolemmal L-type Ca^{2+} channels that are near the jSR. The complex of L-type Ca^{2+} channels (located in the sarcolemma) and the jSR (with its cluster of about 100 RyR2s (Franzini-Armstrong et al., 1999; Soeller et al., 2007) constitute the couplon (Franzini-Armstrong et al., 1999; Stern, 1992). The local-control theory and our current understanding of local Ca^{2+} dynamics increase the importance of knowing about the location, density, and regulation of intracellular ultrastructures (channels, pumps, regulatory proteins, membrane structures, etc.) involved in SR Ca^{2+} cycling.

Intermyofibrillar mitochondria (IMFMs; Fig. 1) span the sarcomere from the couplon at one Z-disk to the couplon at the next Z-disk and are thus "bookended" by the jSR. They are surrounded by the network ("free") SR (nSR) which forms a thin intricate network (rete) from one jSR to another jSR (while interconnected with the entire SR within the cell and to the ER and nuclear envelope (Wu and Bers, 2006)). Additionally, these IMFMs are packed between the nearby myofibrils of the sarcomere that contract with each $[Ca^{2+}]_i$ transient (i.e. global calcium release). The IMFMs are the intracellular organelles (other than the SR) that are positioned closest to the

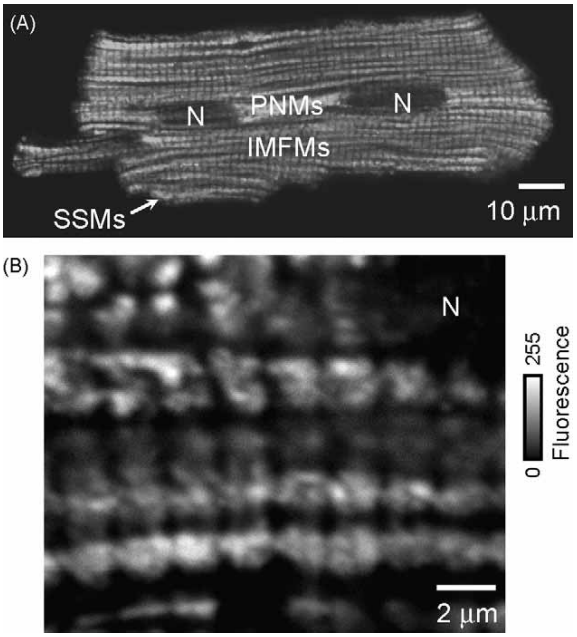

Fig. 1. Location of mitochondria in a rat ventricular myocyte. Immunofluorescent labeling of VDAC as a mitochondrial marker. Two representative cells are shown with lower (A) and higher (B) resolution. IMFMs, intermyofibrillar mitochondria; N, nucleus; PNMs, perinuclear mitochondria; SSMs, subsarcolemmal mitochondria. Adapted from Parfenov et al. (2006).

microdomains of elevated local $[Ca^{2+}]$ during each Ca^{2+} spark, the localized calcium signal from a single jSR (Cheng et al., 1993), or during each $[Ca^{2+}]_i$ transient (Ramesh et al., 1998; Sharma et al., 2000). The major role for the mitochondria is to provide ATP needed for cellular function including contraction and SERCA2a Ca^{2+} pumping (Chen et al., 1996, 1998; Maack and O'Rourke, 2008; Yang and Steele, 2000, 2001). Because of its location and the specific features of its biology and function, another possible mitochondrial function is in the regulation of SR Ca^{2+} cycling. For example, mitochondria appear to play a role in the synthesis of an activator of Ca^{2+} uptake into SR, cyclic ADPR (Lukyanenko et al., 2001a). ADPR cyclase (also known as CD38), which produces two potent Ca^{2+} messengers, cyclic ADPR and NAADP from β-NAD^+, was found to be bound to mitochondrial membranes in a variety of cells including cardiac myocytes (Chini and Dousa, 1995; Franco et al., 1998; Guse, 2000; Meszaros et al., 1997; Mojzisova et al., 2001; Munshi et al., 2000; Lee, 2001; Lee et al., 1997; Liang et al., 1999; Okamoto et al., 2000; Yusufi et al., 2001; Ziegler et al., 1997). Under some conditions, Ca^{2+} release from the SR could be modulated by mitochondrial reactive oxygen species (ROS) (Akar et al., 2005; Wang et al., 2008; Yan et al., 2008; Zorov et al., 2006); however, the most intriguing effect of mitochondria on local Ca^{2+} signaling could be from the possible involvement of mitochondria in the uptake and release of Ca^{2+}, a process we will call "mitochondrial Ca^{2+} cycling". Reports of dynamic fluctuations of mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) vary with respect to the extent and speed of both uptake and release (Brandes and Bers, 2002; Dedkova and Blatter, 2008; Maack et al., 2006; O'Rourke, 2007; Robert et al., 2001; Sedova et al., 2006). Certainly the existence of a favorable electrochemical gradient for passive Ca^{2+} accumulation by the mitochondria (matrix potential is about −180 mV with

respect to the cytosol), a Ca^{2+} permeable channel (the so-called uniporter or MCU) and a mechanism for extrusion (the mitochondrial Na^+/Ca^{2+} exchanger along with the mitochondrial Na^+/proton exchanger) lay the foundation for such possibilities. These possibilities are certainly intriguing and suggest that mitochondrial Ca^{2+} cycling may be important in normal and pathological conditions, but this assessment depends critically on the speed and extent of Ca^{2+} movement across the mitochondria. In this review we discuss data and hypotheses concerning mitochondrial Ca^{2+} movement in ventricular myocytes and review approaches for productive future work.

2. Ultrastructural basis

It has been suggested that Ca^{2+}, released from the endoplasmic reticulum of nonmuscle cells, may be accumulated to some extent by local mitochondria because of the close anatomical relationship between the two organelles (Csordas et al., 2002; Frey and Mannella, 2000; Hajnoczky et al., 2000; Pacher et al., 2002; Rizzuto et al., 1993; Spät et al., 2008; Szalai et al., 2000). Similarly, Ohata et al. (1998) suggested that mitochondrial accumulation of Ca^{2+} released from the cardiac SR could be mediated by the structural association between IMFM and the SR. The details of ultrastructural links, however, remain under investigation.

2.1. Cardiac mitochondria

Three subpopulations of mitochondria in heart are often discussed: IMFMs, subsarcolemmal mitochondria (SSMs) and perinuclear mitochondria (PNMs). The three types are distinguishable by their location, morphology, or by method of isolation. SSMs are readily discernable in skeletal muscle fibers (Rambourg and Segretain, 1980). Originally, the name "subsarcolemmal" referred to all mitochondria that could be easily isolated by polytron treatment of the tissue (Chemnitius et al., 1993; Matlib et al., 1978). In contrast, IMFMs are tightly packed within the cell as described earlier, and can only be isolated after nagarse treatment (Matlib et al., 1978). It was shown that these two groups of cardiac mitochondria (SSMs and IMFMs) are very distinct morphologically and, probably, biochemically from each other (Matlib et al., 1978; Palmer et al., 1977).

Cardiac mitochondria are also classified into PNMs and IMFMs (Fawcett and McNutt, 1969; McNutt and Fawcett, 1969; Segretain et al., 1981). Immunofluorescent labeling (Fig. 1) shows the difference in their shape and size. IMFMs are much larger and have a complicated shape in comparison to the smaller and more rounded PNMs. Fig. 2A shows a micrograph from an ultrathin section through the central part of a cardiac cell. Dissimilarities in shapes, matrix electron densities, and internal membrane extensions between the two mitochondria populations are readily observed. The differences in matrix electron density and in internal membrane extension have been suggested to reflect differences in functional activity between the two types of mitochondria (Fawcett, 1966; Hackenbrock, 1968; Kononova, 1982). PNMs usually appear circular on electron micrographs, while IMFMs appear oval and occupy the entire space between Z-lines (Fawcett and McNutt, 1969). Fig. 2B shows that the tightly packed IMFMs are located in very close proximity to known Ca^{2+} release site, the jSR, which is located between the IMFM and sarcolemma of T-tubule (TT).

Questions regarding the shape and size of cardiac mitochondria are straight forward (Aon et al., 2006). Quenching of ethylrhodamine fluorescence by a narrow laser beam revealed several mitochondrial clusters within one cardiomyocyte (Amchenkova et al., 1988), each of which could represent a single branched mitochondrion. Indeed, cardiac mitochondria were shown to branch, bend back upon themselves, or have two lobes connected by narrower segments (Fawcett and McNutt, 1969; Segretain et al., 1981).

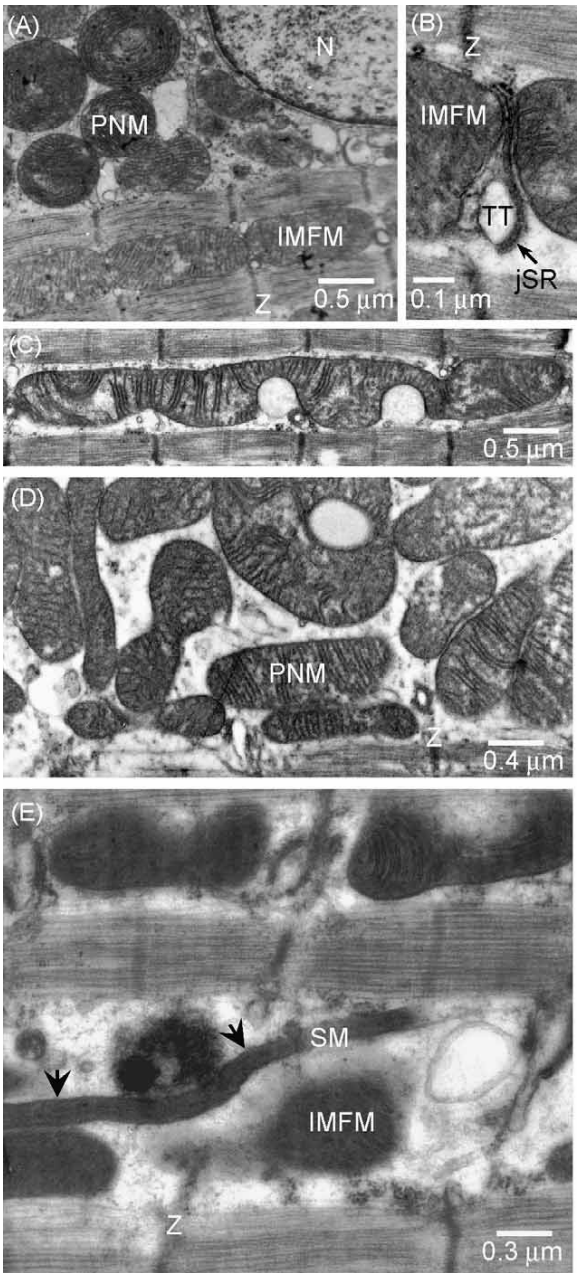

Fig. 2. Types of cardiac mitochondria in a rat ventricular myocyte. Electron micrograph; longitudinal ultrathin section, conventional fixation. Longitudinal ultrathin section. (A) Perinuclear and intermyofibrillar mitochondria. (B and C) Intermyofibrillar mitochondria. (D) Perinuclear mitochondria. (E) Intermyofibrillar and slender mitochondria. jSR, junctional sarcoplasmic reticulum; Z, Z-line; IMFM, intermyofibrillar mitochondrion; N, nucleus; PNM, perinuclear mitochondrion; SM, slender mitochondrion; TT, T-tubule; black arrows show regions where four membranes are clearly seen Lukyanenko et al. (unpublished data).

Fig. 2C and D shows that one IMFM (Fig. 2C) could span four sarcomeres (8 μm) in length, and that PNMs could be far from round (Fig. 2D).

Although the morphology of the three mitochondria groups is dynamic and varied (Rube and van der Bliek, 2004), there are also two rarely observed groups of cardiac mitochondria: slender mitochondria (SM) and nuclear mitochondria. SM are very long mitochondria or mitochondrial branches, though both extremities of the mitochondrion have not yet been imaged. A typical SM is

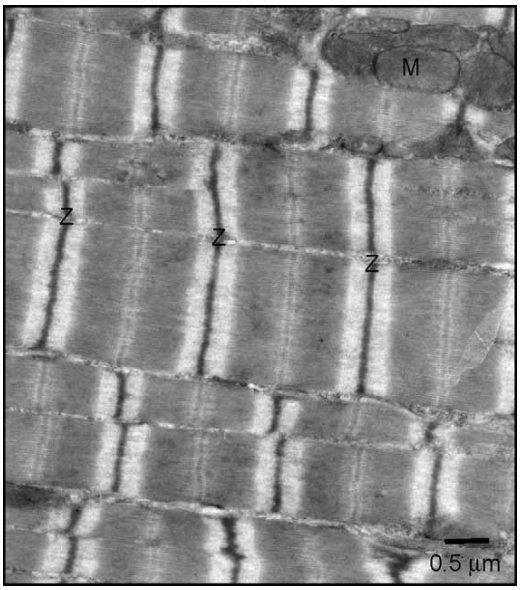

Fig. 3. Ultrastructure of ventricular cell from a rat suffering from heart failure. Heart failure was induced with isoproterenol (3rd week; 0.3 mg/kg injections). *Note*: mitochondrial regressive changes and abnormal I bands. Left ventricle; conventional microwave fixation. M, mitochondrion; Z, Z line. Adapted from Lukyanenko (2007).

shown in Fig. 2E. Arrowheads indicate regions where four membranes are clearly visible. The continuity of these mitochondria makes them ideal candidates for what was described in classical studies by Fawcett and McNutt (1969) as "slender lateral or longitudinal prolongations about 0.1 μm in diameter and of variable length". Nuclear mitochondria in cardiac myocytes were described recently (Skulachev et al., 2004), but only in end-stage cardiac failure.

During heart failure, mitochondria undergo changes and IMFMs may lose their connections to the cytoskeleton and SR (Jones et al., 1975; Su et al., 2000). Fig. 3 shows a significant reduction in the density of IMFMs and abnormally small mitochondria in a ventricular cell from a rat suffering from heart failure (compare with Fig. 2). Recently, we demonstrated that under normal (i.e. physiological) conditions the mitochondrial outer membrane is very close to the surrounding structures, including the SR and other mitochondria (Fig. 4) (Lukyanenko et al., 2007; Parfenov et al., 2006; Salnikov et al., 2007). The packing was largely inaccessible even to 3 nm particles, and the molecular details of this organization remain largely unknown. Some data suggest a role for desmin and tubulin in maintaining structural integrity (Watkins et al., 1987). Milner et al. (1999, 2000) showed that desmin-null (desmin −/−) cardiac myocytes have subsarcolemmal mitochondrial clumping and reduced IMFMs, although these observations require further quantitative and physiological investigation. The manner in which desmin could be connected to the outer mitochondrial membrane (OMM) is unclear; however it could be similar to adhesive structures described for bacteria (Knight et al., 2000; Mootha et al., 2003; Paschen et al., 2003; Sauer et al., 2000).

Tubulin was reported to establish tight contacts to the voltage-dependent anion channel (VDAC) (Carré et al., 2002; Monge et al., 2008; Rostovtseva and Bezrukov, 2008; Rostovtseva et al., 2008). In addition to tubulin, another connective candidate is mitofusin. Mitofusin was shown to tether the endoplasmic reticulum to mitochondria in mouse embryonic fibroblasts and HeLa cells (Brito and Scorrano, 2008). Most recently, García-Pérez et al. (2008) reported a very specific physical coupling between the OMM and SR in cardiac cells. This hypothesis was based on the existence of direct Ca^{2+}

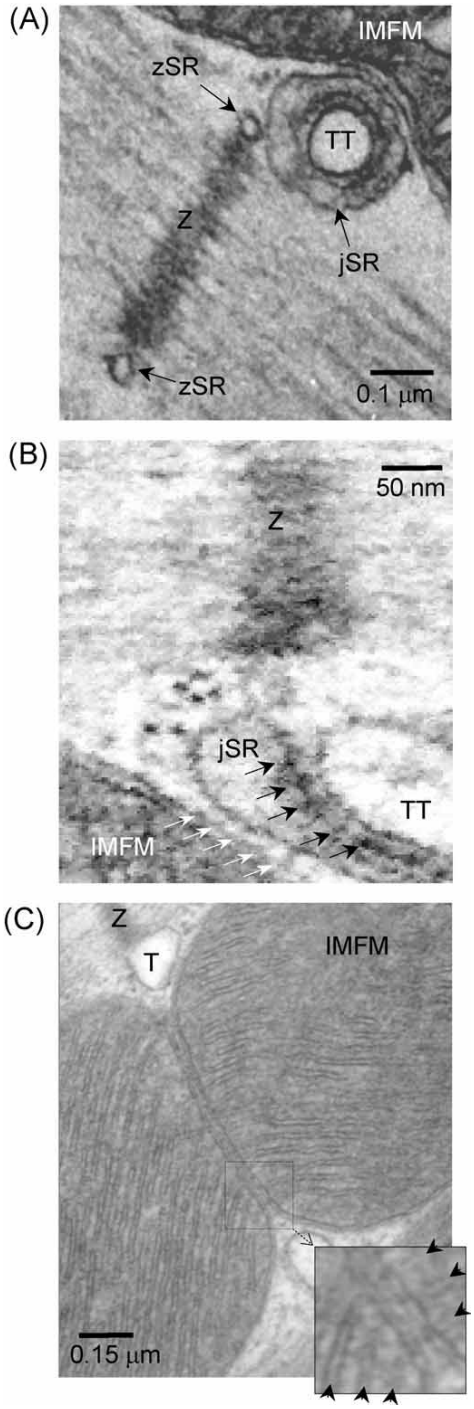

Fig. 4. Mitochondrial contacts in a rat ventricular myocyte. (A and B) The electron micrographs show areas of contact between a mitochondrion and T-tubule. (C) Intermitochondrial contact. Inset shows the ultrastructural organization of the contact. Arrowheads show mitochondrial cristae. IMFM, intermyofibrillar mitochondrion; jSR, junctional SR; M, mitochondrion; TT, T-tubules; zSR, z-tubules of the SR; Z, Z line (Z disk); black arrows show RyRs; white arrows show structures connecting IMFM and jSR. Adapted from Parfenov et al. (2006).

channeling from the SR to the mitochondrial matrix. The physiological or pathophysiological context of this hypothesis is yet to be elucidated.

2.2. Are IMFMs different from other cardiac mitochondria?

Cardiac IMFMs have been isolated from a number of animal species (Hoppel et al., 1982; Matlib et al., 1978; McMillin-Wood et al., 1980; Ohata et al., 1998; Palmer et al., 1977; Weinstein et al., 1985, 1986). The abundance of fibrillar material in the heart, coupled with the tight packing of mitochondria between the Z-disks, makes isolation of IMFMs very difficult. Electron microscopy of the corresponding pellets showed that the polytron preparation was practically unable to extract IMFMs. Therefore, to release IMFMs, a nagarse preparation was used. Once isolated, IMFMs were shown to have different biochemical properties than other cardiac mitochondria types. Compared to the polytron-isolated mitochondria, IMFMs have: (1) up to three-times higher rates of Ca^{2+} uptake and up to two-times lower K_m values; (2) 50% higher rates of oxidative phosphorylation, and (3) significantly higher cytochrome content (Matlib et al., 1978; McMillin-Wood et al., 1980; Palmer et al., 1977). IMFMs were shown to be less vulnerable to global ischemia (Weinstein et al., 1985), while cardiomyopathy leads to mitochondrial oxidative defects confined wholly to the IMFMs (Hoppel et al., 1982), though possible damage to IMFMs by nagarse may invalidate these data.

These dissimilarities between cardiac mitochondrial subpopulations were confirmed, to some extent, in intact mitochondria by Kononova (1982). In these experiments, hypoxia was followed by quantitative analysis of changes in mitochondrial ultrastructure. Hypoxia resulted in the swelling of all cardiac mitochondria. However, after a 1-day period, only IMFMs and PNMs showed a significant increase in area and number of cristae, and only PNMs significantly (almost double) increased in number, thereby suggesting different biochemical properties in the studied mitochondrial subpopulations.

Reports presented by Isenberg et al. (1993) and Gallitelli et al. (1999) supported this hypothesis. They described experiments in which isolated guinea-pig ventricular myocytes were instantly frozen with supercooled propane (−196 °C) during stimulation with paired voltage or current clamp depolarizing pulses. Cell contact with the coolant induced a negative spike followed by a large positive current. The position of this artifact enables temporal resolution of the freezing incident. Using electron beam microanalysis it was determined that during systole, the Ca^{2+} increase in peripheral mitochondria was at least three times that of the central mitochondria. However, although the analyzing beam had a diameter of 16 nm (Isenberg et al., 1993), it was uncertain whether it was specifically focused on the mitochondrial matrix, given that the ultrastructure of the mitochondria, more specifically the deeply infolded cristae, does not permit distinction between Ca^{2+} inside the matrix and Ca^{2+} in the mitochondrial intermembrane space. Therefore, the data may reflect changes in Ca^{2+} within both the mitochondrial intermembrane space and in the matrix. This provides a plausible explanation as to why Ho et al. (2003) did not detect any increase in IMFM Ca^{2+} during contractions. It should be noted, however, that the approach used by Ho et al. (2003) was less precise than that of Isenberg et al. (1993).

2.3. Junctional sarcoplasmic reticulum

SR Ca^{2+} cycling is a balance between Ca^{2+} release and uptake (Eisner et al., 2000), and under steady-state conditions these fluxes must be equal. Electron microscopy and immunolabeling are common visualization tools used to study the localization and membrane structures of Ca^{2+} transport proteins. The intracellular complexes primarily responsible for Ca^{2+} release and uptake during the cardiac cell contractile cycle are the couplons (Frank, 1990; Franzini-Armstrong et al., 1998, 1999; Gathercole et al., 2000; Jorgensen et al., 1982, 1993; Yang et al., 2002). The jSR located in close proximity to the TT is seen as a pancake that is wrapped around the TT with wispy connections to the network SR (Brochet et al., 2005). A cross-sectional view of the jSR reveals two membrane surfaces (Fig. 4A and B): one facing the TT sarcolemma studded with "feet" (i.e. RyR2 homotetramers), and the other (rich in SERCA2a) facing the mitochondrial outer membrane (Jorgensen and Jones, 1987; Jorgensen et al., 1982). The jSR is equidistant (∼15 nm) from the sarcolemma and the IMFM outer membrane. These jSR membranes were shown to contain major structural components of the SR Ca^{2+} cycling system (Jorgensen and Jones, 1987; Jorgensen et al., 1982; Ozawa et al., 1976; Sommer and Spach, 1964).

Recently, we have developed a practical approach to measure the functional distances between membranes *in vivo* (Lukyanenko, 2007; Parfenov et al., 2006; Salnikov et al., 2007). Our experiments revealed that even during contraction, gold nanoparticles as small as 3 nm in diameter could not enter the space between the jSR and the membranes (Parfenov et al., 2006). The full molecular and biophysical explanation for this observation involves the consideration of the many proteins and molecular structures that may fill those spaces.

The distribution of sarcolemmal Ca^{2+} channels, exchangers, and pumps is critical to the understanding of Ca^{2+} signaling. DHPRs are located in the TT and face the "subspace" (or junctional cleft), which separates the TT and the jSR. The sarcolemmal Na^+/Ca^{2+} exchanger proteins are found in both the exterior sarcolemma and the TT sarcolemma but not in the junctional cleft (Frank et al., 1992; Kieval et al., 1992; Scriven et al., 2005). Little is known about the function of the IMFM outer membrane, facing the Ca^{2+} uptake proteins of the jSR, located in close proximity to the Z-line network SR which is thought to be rich in SERCA2a (Jorgensen et al., 1982; Ozawa et al., 1976; Prestle et al., 2003).

3. Mechanisms of Ca^{2+} cycling

Our purpose here is to focus on the interplay between mitochondrial and SR Ca^{2+} signaling. Therefore, other aspects of membrane (sarcolemmal) Ca^{2+} cycling will not be discussed. See Bers (2001) for detailed review on this topic.

3.1. Sarcoplasmic reticulum Ca^{2+} cycling

In mammalian cardiac myocytes, the SR serves as the intracellular Ca^{2+} store. It amplifies the "trigger" Ca^{2+} that enters across the sarcolemma to produce the $[Ca^{2+}]_i$ transient which underlies cardiac contraction (Bers, 2001, 2002a,b; Chiesi et al., 1994; Cannell et al., 1995; Feher and Fabiato, 1990). The amount of Ca^{2+} in the SR lumen ($[Ca^{2+}]_{SR}$) depends on the functional state of SR Ca^{2+} uptake and Ca^{2+} release mechanisms. Cytoplasmic Ca^{2+} itself is the main regulator of Ca^{2+} release from the SR in cardiac myocytes (Bers, 2001, 2002a,b; Fabiato, 1985, 1992; Feher and Fabiato, 1990). Ca^{2+} release and re-uptake are highly coordinated through changes in $[Ca^{2+}]_{SR}$ and also depend on the $[Ca^{2+}]$ gradient across the SR membrane (Bhogal and Colyer, 1998; Ching et al., 2000; Fabiato, 1992; Györke and Györke, 1998; Ikemoto and Yamamoto, 2000; Lukyanenko et al., 1996, 1998, 2001b; Sitsapesan and Williams, 1995). SERCA2a, the SR Ca^{2+} pump, maintains the Ca^{2+} gradient between the cytosol and the SR lumen ($[Ca^{2+}]_{SR}/[Ca^{2+}]_{cyt}$ = ∼15,000), using the free energy available from hydrolysis of ATP (ΔG_{ATP} = ∼60 kJ/mol). There is a tight coupling between the SR Ca^{2+} gradient and the ΔG_{ATP} (Chen et al., 1996, 1998). As Ca^{2+} accumulates in the lumen the off-rate of Ca^{2+}

from SERCA2a may become the rate-limiting step, and Ca^{2+} pumping decreases through "back-inhibition" (Pozzan et al., 1994). The details of these events clearly depend on the efficiency of transport, its stoichiometry (how many Ca^{2+} ions per ATP consumed) and other features of the pump. Intraluminal Ca^{2+} can also modulate the activity of SERCA2a by modulating the activity of protein kinases that interact with the luminal complex of SERCA2a (Bhogal and Colyer, 1998). The efficiency of transport by SERCA2a in both physiological and pathophysiological conditions (such as ischemia) may be at 75–85% of the theoretical thermodynamic limit on the basis of the ΔG_{ATP}, leaving the possibility of kinetic and further thermodynamic regulation (Chen et al., 1998; Feher and Fabiato, 1990). Overall, the activity of SERCA2a is not only dependent on the energy state of the cell but can also be kinetically regulated by SR proteins, protein kinases, and by phospholamban (PLN) (Bers, 2001). For instance, removing the kinetic limitation of PLN on the activity of the SERCA2a allows the SR Ca^{2+} gradient to move closer to its thermodynamic limit (Chen et al., 1998). Exactly what the thermodynamic limit is, however, is not precisely known.

Failure in the control mechanisms of SR Ca^{2+} cycling leads to a variety of cardiac dysfunctions. Spontaneous Ca^{2+} release and increased SR Ca^{2+} "leak" have been implicated in cardiac dysfunctions such as genetic and acquired triggered arrhythmias and the initiation of ventricular fibrillation during postischemic reflow (Bellinger et al., 2008; Carmeliet, 1999; Ferrier, 1976; Ishide, 1996; Janse, 1999; Kihara and Morgan, 1991; Lakatta, 1992; Lehnart et al., 2006, 2008; Marks, 2001; Pogwizd and Bers, 2002). Defective SR Ca^{2+} cycling was found to be responsible for defective excitation–contraction coupling in heart failure (Currie and Smith, 1999; Haghighi et al., 2001; Hasenfuss and Pieske, 2002; Hobai and O'Rourke, 2001; Kirchhefer et al., 1999; Lehnart et al., 2006, 2008; Maier and Bers, 2007; Schmidt et al., 1998).

3.2. Ca^{2+} sparks

A Ca^{2+} spark is a fluorescent signal corresponding to the localized release of Ca^{2+} from a jSR cluster of RyR2s. The Ca^{2+} spark has a time to peak of about 10 ms with a size (full-width at half of the maximum level) of about 2 μm and a volume at that time of about 10 fl. It is thought to represent the efflux of Ca^{2+} from a RyR2 cluster (average size is about 100 RyR2s (Franzini-Armstrong et al., 1999; Soeller et al., 2007)). While the exact number of RyR2s that are involved in the Ca^{2+} spark is not precisely known, there is reason to believe that it could involve all of the channels in the cluster, but a variable fraction could also be involved with little difference in Ca^{2+} spark characteristics (Cheng and Lederer, 2008; Sobie et al., 2002). The Ca^{2+} spark is the elementary event of SR Ca^{2+} release (Cheng et al., 1993; Györke et al., 1997; Guatimosim et al., 2002; Lopez-Lopez et al., 1995; Lukyanenko et al., 2000, 2007). Ca^{2+} sparks can occur spontaneously, or can be evoked by the activation of sarcolemmal L-type Ca^{2+} channels (Cannell et al., 1994; Lopez-Lopez et al., 1995; Wang et al., 2001). Under normal conditions, nearly all of the spontaneous or diastolic Ca^{2+} sparks remain localized and do not activate nearby (1 μm away) Ca^{2+} spark sites (jSR) (Cheng et al., 1993, 1996; Lukyanenko and Györke, 1999; Lukyanenko et al., 1996, 1999). Under conditions of increased SR Ca^{2+} load, Ca^{2+} sparks increase in amplitude and frequency and become initiation sites of propagating Ca^{2+} waves (Cheng et al., 1993, 1996; Izu et al., 2001; Lukyanenko and Györke, 1999; Lukyanenko et al., 1996). With respect to our discussion of mitochondria, Ca^{2+} sparks are of comparable size to a IMFM and originate in close proximity to IMFMs. Ca^{2+} sparks can be readily influenced by changes in local $[Ca^{2+}]_i$ and are clearly an excellent tool to use in the study of crosstalk between the IMFM and the SR.

Ca^{2+} sparks have been recorded in close proximity to IMFMs and PNMs (Cheng et al., 1996; Lukyanenko et al., 2007; Shacklock et al., 1995; Yang and Steele, 2005). Recently we showed that the spatiotemporal characteristics of sparks found around PNMs and around IMFMs are very similar with respect to many parameters. However, Ca^{2+} sparks from the PNM zones were significantly longer in duration (Lukyanenko et al., 2007) than the usual diastolic Ca^{2+} sparks. Our data suggested that the RyR2 clusters that produce stereotype Ca^{2+} sparks are likely to be similar in structure. We speculate that the differences in Ca^{2+} spark duration could be due to the functional differences in nearby mitochondria, but more experiments are needed to verify the observation and better characterize it.

3.3. Mitochondrial Ca^{2+} cycling

In adult ventricular myocytes, mitochondria occupy 30–40% of the intracellular volume, presumably reflecting the huge demands of the contractile machinery for ATP production (Maack and O'Rourke, 2008). Under normal conditions, the very negative inner mitochondrial membrane (IMM) potential (∼−180 mV relative to the cytosol) provides a strong electrochemical driving force for Ca^{2+} to enter the mitochondrial matrix from the cytosol (Fig. 5). It is thought that the pathway by which Ca^{2+} crosses the IMM is the MCU

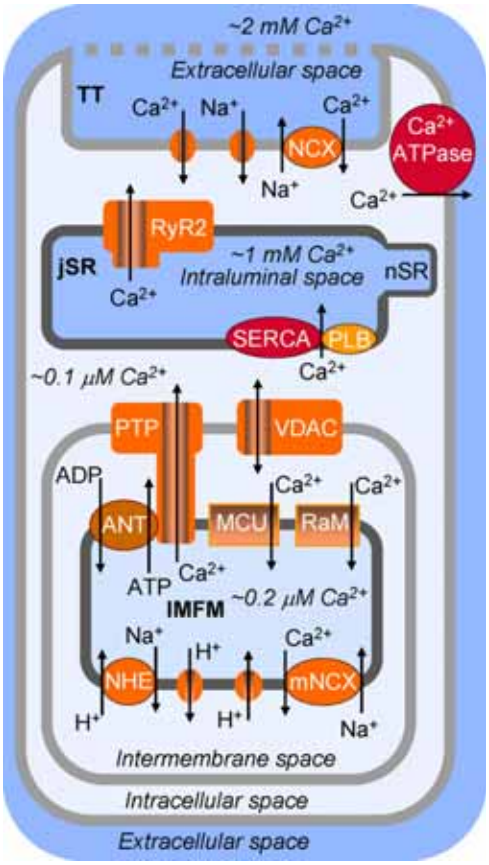

Fig. 5. Mitochondrial Ca^{2+} cycling in ventricular myocytes. Schematic of local Ca^{2+} cycling in ventricular cardiac myocytes: sarcolemmal, SR, and mitochondrial Ca^{2+} cycling. This schematic is a conceptual representation of the location of structures involved in the local interplay of IMFM, SR, and sarcolemmal Ca^{2+} cycling. ADP, adenosine diphosphate; ANT, adenine nucleotide translocase; ATP, adenosine trisphosphate; junctional sarcoplasmic reticulum, MCU, electrogenic mitochondrial Ca^{2+} uniporter; mNCX, Na^+/Ca^{2+} exchanger; NHE, Na^+/H^+ exchanger; nSR, network SR; PTP, permeability transition pore; RaM, rapid mode of Ca^{2+} uptake; TT, transverse tubule; VDAC, voltage-dependent anion channel.

(Dedkova and Blatter, 2008; Gunter and Pfeiffer, 1990; O'Rourke, 2007; Robert et al., 2001; Sedova et al., 2006). The molecular identity of the uniporter remains uncertain (Kirichok et al., 2004) and hence, the characteristics of its conductance, kinetics and regulation remain largely unknown and untested. Even more obscure is a faster mode of mitochondrial Ca^{2+} uptake known as rapid uptake mode (RaM), which has been described as a rapid self-inhibitory Ca^{2+} uptake with a recovery period of ~60 s (Buntinas et al., 2001; Sparagna et al., 1995).

At steady-state, the Ca^{2+} influx into the mitochondria must be balanced by an efflux. However, as noted above mitochondrial Ca^{2+} dynamics are still both uncertain and controversial. The efflux of Ca^{2+} is believed to depend on mitochondrial Na^+/Ca^{2+} exchanger (mNCX) and Na^+/proton exchanger (NHE). It has been suggested that the mNCX could extrude Ca^{2+} from the matrix as long as the Na^+ that enters the matrix also has a way to exit. This has been thought possible by the NHE with $K_{0.5}$ ~4–8 mM (Bers et al., 2003; Cox and Matlib, 1993; Dash and Beard, 2008; Fry et al., 1984b; Saotome et al., 2005). Other possible contributors to the mitochondrial Ca^{2+} flux include the permeability transition pore (PTP, the molecular and functional characteristics of which are also uncertain and controversial) and a putative H^+/Ca^{2+} exchanger (Hüser and Blatter, 1999; Kang et al., 2007; Nicholls and Chalmers, 2004; Rizzuto et al., 2000), but the details remain murky. As $[Ca^{2+}]$ in mitochondria ($[Ca^{2+}]_m$) increases with time, Chalmers and Nicholls (2003) suggest that there may be three phases of mitochondrial Ca^{2+} accumulation. First, the modest increase in $[Ca^{2+}]_m$ influences enzyme function (e.g. matrix dehydrogenase). Second, as $[Ca^{2+}]_m$ increases further, the mitochondria may serve to buffer $[Ca^{2+}]_i$. Third, when $[Ca^{2+}]_m$ becomes even higher, the PTP may be activated. Exactly how these elements may interact with physiological Ca^{2+} extrusion or with the hypothesized mitochondrial Ca^{2+}-activated K^+ channels (mBK_{Ca}) (Kang et al., 2007) will be model dependent and the answer awaits critical new experiments. There continues to be related vexing questions about many aspects of mitochondrial Ca^{2+} regulation.

Due to its dependency on the mitochondrial inner membrane potential, mitochondrial Ca^{2+} entry and exit should be affected by everything that may affect the potential across the mitochondrial inner membrane. This should included pH, P_i, ADP, ATP, $[Na^+]_i$ and $[Ca^{2+}]_i$ (Cortassa et al., 2003; Dedkova and Blatter, 2008; Nicholls and Crompton, 1980; Oliveira and Kowaltowski, 2004). Under normal conditions, with the simple models put forward so far, the extrusion of Ca^{2+} from the mitochondria should depend primarily on $[Na^+]_{mito}$ but also on $[Na^+]_i$. Thus, mNCX is thought to be the primary Ca^{2+} release mechanism under physiological conditions (Gunter et al., 1994; Rizzuto et al., 2000), however its capacity and kinetics remain uncertain. The mNCX is pharmacologically and molecularly distinct from sarcolemmal Na^+/Ca^{2+} exchanger. For example, the diltiazem analogue, benzothiazepine CGP 37157, has been reported to inhibit mNCX (Baron and Thayer, 1997; Cox and Matlib, 1993; Cox et al., 1993; White and Reynolds, 1997), while diltiazem itself inhibits Ca^{2+} channels and not the sarcolemmal Na^+/Ca^{2+} exchange (Kuo et al., 2002; Watano et al., 1999).

The PTP, a large non-selective conductance pore, appears to be regulated by $[Ca^{2+}]_m$ (Bernardi et al., 1994; Haworth and Hunter, 1979; Kroemer et al., 2007; Szabadkai and Duchen, 2008; Zoratti and Szabo, 1995). Recently such a Ca^{2+}-induced permeability transition was demonstrated in cardiac mitochondria (Kang et al., 2007; Salnikov et al., 2007), but the totality of the data is not compelling. Activation of the putative PTP collapses the membrane potential and can release Ca^{2+} through the pore itself and/or may allow Ca^{2+} efflux via reversal of the MCU (Fiskum and Cockrell, 1985; Kang et al., 2007; Pacher and Hajnoczky, 2001), but these possibilities remain largely speculative. The most potent inhibitor of the PTP in cardiac cells is cyclosporin A (CsA) (Rizzuto et al., 2000).

The PTP (as we understand it to exist) permits molecules as large as 1500 Da to pass. The exact molecular composition of the PTP remains uncertain, however. It is argued that the PTP has a component in the OMM (e.g. the voltage-dependent anion channel, VDAC) and another component in the IMM (e.g. ANT, the adenine nucleotide transporter), and appears to be regulated by cyclophilin D in the IMM. The outer mitochondrial membrane is a minimal barrier for small molecules because it contains VDAC, a channel that permits both anions and cations as well as uncharged substances to pass; VDAC allows non-electrolytes up to ~5000 Da to permeate, and has been referred to as "mitochondrial porin" (Colombini, 1987, 2004; Crompton, 1999; Gincel et al., 2001; Kroemer et al., 2007; Murphy and Steenbergen, 2007; Rostovtseva et al., 2002a,b, 2005; Szabadkai and Duchen, 2008). The VDAC pore does not prevent passage of calcium ions even when VDAC is "closed", while passage of molecules as big as ATP is inhibited (Rostovtseva et al., 2005). The involvement of VDAC in mitochondrial Ca^{2+} signaling is complex. For example, despite its low selectivity, VDAC was reported to exhibit Ca^{2+}-dependent regulation (Gincel et al., 2001; Shoshan-Bormatz et al., 2003; but see Rostovtseva et al., 2005).

Other mitochondrial Ca^{2+} permeation paths have also been reported. Shey-Shing Sheu's group found RyRs type one (RyR1) in the mitochondrial membrane (Beutner et al., 2001; Sharma et al., 2000). The existence of RyR1s in the mitochondrial inner membrane was recently supported by additional work from this group (Altschafl et al., 2007). However, due to the existence of Ca^{2+} microdomains at the ends of the intermyofibrillar mitochondria described above, there is high probability that SR membranes may contaminate mitochondrial membrane preparations despite the great care used in their preparation (Taylor et al., 2003). It was, however, RyR1 that was found not the normal cardiac type 2 isoform. This issue was recently reexamined by Spät et al. (2008) who concluded that mitochondria in the rat heart are highly resistant to purification from SR membranes. Our own experiments involving the use of anti-bodies to all types of RyRs (Salnikov et al., 2005) failed to reveal any RyRs at the center of mitochondria as expected, assuming that the entire IMM is available to RyR1. This work however, did not rule out the possibility that they could be located at the connections between the inner and outer mitochondrial membranes and therefore undetectable by immunogold labeling, which with two IgGs, can have an error margin of up to 20 nm.

Recently, reports of "Ca^{2+} channeling" from the SR to the mitochondrial matrix have emerged (García-Pérez et al., 2008; Spät et al., 2008). These reports suggest that in cardiac myocytes, the SR RyR2s could be located in close proximity to the OMM, and could provide a sufficient Ca^{2+} trigger for induction of mitochondrial membrane permeabilization allowing the transfer of Ca^{2+} into the cardiac mitochondria (García-Pérez et al., 2008). García-Pérez et al. (2008) also suggested that mitochondrial type 1 RyRs reported earlier (Altschafl et al., 2007) could be involved in coupling between the SR and OMM. To date no compelling functional linkage between either SR or ER and mitochondria has been provided. In 2001, Kaasik et al. showed the possible existence of direct adenine nucleotide channeling between the cardiac jSR and IMFM. The provocative hypothesis that an array of "direct connections" between the SR/ER and the mitochondrial matrix exists cannot be readily disproved and the data to date are, at best, suggestive. Additional unambiguous experiments are needed to test the hypothesis. Considering the highly restricted space between the SR and IMFM (Lukyanenko et al., 2007; Parfenov et al., 2006; Salnikov et al., 2007), it can be concluded that regions of close apposition or contact between mitochondria and the SR/ER are likely to be important. However, direct evidence of the involvement of mitochondria in cardiac Ca^{2+} signaling remains largely absent. The role(s) of mitochondrial $[Ca^{2+}]_m$

fluctuations with the local Ca^{2+} sparks or the cell-wide $[Ca^{2+}]_i$ transients remains intriguing and provocative (Maack et al., 2006; O'Rourke, 2007).

The dynamics of the reported SR-IMFM contacts and the time-dependent changes in quantitative morphometry must be elucidated to provide support for putative SR-IMFM crosstalk. At this point we can only speculate that the systolic increase in $[Ca^{2+}]_i$ (that could be higher locally) may be important. Many questions are raised regarding these matters. If present, could crosstalk affect IMFM dependent apoptosis (Pan et al., 2001)? How do local and global $[Ca^{2+}]_i$ affect changes in cytochrome *C* and relocation of Bax (a Bcl-2 family member) from the cytoplasm to the IMFM outer membrane (Heiskanen et al., 1999; Pan et al., 2001)? Can these proteins form aggregates with VDAC in the mitochondrial outer membrane (Godlewski et al., 2002; Kluck et al., 1999; Kuwana et al., 2002)? If so, what are the consequences?

4. Interplay between mitochondrial and sarcoplasmic reticulum Ca^{2+} signals

4.1. Experimental data

There are two aspects of SR Ca^{2+} signaling that could be influenced by the mitochondria under normal physiological conditions. During systole the mitochondria are bathed by the very high $[Ca^{2+}]_i$ at their ends near the jSR and the global $[Ca^{2+}]_i$ in the middle. They compete (albeit poorly) with the SR for uptake of Ca^{2+}. The efflux of Ca^{2+} from the mitochondria during diastole must equal the influx acquired during systole (on average and in the steady-state). To the extent that there is Ca^{2+} influx during systole, the mitochondria should produce an efflux during diastole. Depending on the amount and rate of Ca^{2+} efflux from the mitochondria, there may be a measurable effect on $[Ca^{2+}]_i$. If the efflux is high and focused near the jSR, it may "bias" the local jSR Ca^{2+} signal and influence the probability of the RyR2s to be triggered by the L-type Ca^{2+} channel current influx. If, however, the efflux is low and spread out in space and time, it may have no significant effect. Until the late 1970s, mitochondria were considered an important structure in the control of Ca^{2+} homeostasis (Bers, 2001; Pozzan et al., 1994). However, later it was shown that the MCU only became activated to appreciable levels when $[Ca^{2+}]$ rose above 0.5 μM (Fry et al., 1984a; Pozzan et al., 1994; Sedova et al., 2006) with $K_{0.5}$ ~4–10 μM (Bassani et al., 1998; Sedova et al., 2006). The cellular $[Ca^{2+}]_i$ peaks at about 1 μM, although some regions of each mitochondrion presumably experience a higher concentration. Importantly the conductance of the MCU (i.e. the effective turnover rate) is thought to be quite low (Kirichok et al., 2004), and the density of the uniporters in the IMM is unknown. If the local $[Ca^{2+}]_i$ at the jSR end of the IMFM were to reach 3 μM the nearby MCUs would be significantly activated (García-Pérez et al., 2008). However, the MCUs in the middle of the IMFM would be bathed with a lower $[Ca^{2+}]_i$. Experiments with cardiomyocytes (Bassani et al., 1992, 1993; Bowser et al., 1998; Brandes and Bers, 2002; Duchen, 2000; García-Pérez et al., 2008; Isenberg et al., 1993; Martin et al., 1998; Ohata et al., 1998; Pacher et al., 2000; Pitter et al., 2002; Robert et al., 2001; Sedova et al., 2006; Sharma et al., 2000; Sheu and Sharma, 1999; Szalai et al., 2000; Territo et al., 2001a,b) and other cells types (Arnaudeau et al., 2001; Connor, 1993; Haak et al., 2002; Isaeva and Shirokova, 2003; Isaeva et al., 2005; Jouaville et al., 1995; Maack and O'Rourke, 2008; Spät et al., 2008; Wang and Thayer, 2002) do suggest that the $[Ca^{2+}]_m$ changes with time and reflects the sarcomeric $[Ca^{2+}]_i$ gradient, but this $[Ca^{2+}]_m$ is not calibrated. For example, it was reported that free $[Ca^{2+}]$ in mitochondria under physiological conditions is ~100 nM (Miyata et al., 1991). This is a level that is about the same as the measured $[Ca^{2+}]_i$. However it was also suggested that free $[Ca^{2+}]_m$ may increase during systole to ~700 nM (Brandes and Bers, 2002; Miyata et al., 1991; Ohata et al., 1998). The actual normal change in total mitochondrial Ca^{2+} content under physiological conditions is not known but could readily exceed the 1 mM Ca^{2+} measured by Isenberg et al. (1993).

Recently, the role played by Na^+ in mitochondrial Ca^{2+} regulation was demonstrated in vascular endothelial cells (Sedova and Blatter, 2000), cortex neurons (Raiteri et al., 2002) and cardiac myocytes (Bers et al., 2003; Maack and O'Rourke, 2008; Maack et al., 2006; Sedova et al., 2006). In the cardiac cell, during an action potential, the $[Na^+]$ in the region closest to the membrane was estimated to increase to as high as 80 mM within milliseconds (Gallitelli et al., 1999). While unverified, if the Gallitelli estimate of this increase in $[Na^+]$ during I_{Na} in a narrow subcellular microdomain were supported and applied to the mitochondrion, it may be sufficient to power significant Ca^{2+} efflux from the mitochondria (Piacentino et al., 2003). For many reasons, however, this number is unreasonably high (Lederer et al., 1990). The pressing questions are how fast Ca^{2+} can be released from the IMFM and how much? Using patch clamp and electron probe microanalysis, Isenberg et al. (1993) reported that the peak total $[Ca^{2+}]$ in mitochondria could diminish from 1.0–~0.5 mM in just 50 ms. As an isolated event, this Ca^{2+} efflux from a mitochondrion would produce a 36 nM elevation in a 10 fl volume (assuming a rectangular shaped mitochondrion of 0.2 μm × 0.2 μm × 1.8 μm if the cytosolic Ca^{2+} buffering power were 100). This would be visible using our current methods, unless it were blurred by overlapping $[Ca^{2+}]_i$ signals such as Ca^{2+} sparks. Such Ca^{2+} release events, if they did contribute, would not add any net Ca^{2+} to the signal, instead they would alter the kinetics of the $[Ca^{2+}]_i$ transient. Do note that the condition that triggers SR Ca^{2+} release (the AP) and thus underlie high mitochondrial Ca^{2+} uptake is the same condition that favors mitochondrial Ca^{2+} extrusion (high local $[Na^+]$). Therefore, there are many details that must be addressed both experimentally and with respect to mitochondrial Ca^{2+} modeling before any firm conclusions can be drawn.

4.2. Mathematical models

As noted above, the precise details of SR and mitochondrial Ca^{2+} interplay remain obscure experimentally and theoretically. Since the experimental findings are inconsistent, there is much room for speculation. Mathematical models provide us with the ability to study Ca^{2+} transport in each system in isolation and enable us to refine our experiments or the analysis associated with them. Mathematical models have been used to gain insights into the regulation of energy metabolism in the mitochondria (Jafri et al., 2001; Lambeth and Kushmerick, 2002; Magnus and Keizer, 1998a,b; Nguyen et al., 2007; Tornheim, 1979), Ca^{2+} cycling in the SR (Greenstein et al., 2006; Greenstein and Winslow, 2002; Shannon et al., 2000, 2002, 2004), and the "supply meets demand" phenomena in cardiomyocytes (Cortassa et al., 2006; Nguyen and Jafri, 2005). These models are constructed in a modular fashion, where each module is a detailed kinetic model of the individual elements (i.e. enzymes, other proteins) that constitute the system. While models of Ca^{2+} cycling in the SR and cytosol are abundant, models of Ca^{2+} cycling in the mitochondria are scarce. The few models that have been developed are constrained by parameters largely derived from experiments conducted on isolated mitochondria preparations. In this section we briefly discuss several of these models along with their respective contributions.

One of the most extensive efforts to model mitochondrial Ca^{2+} handling, and its effect on energy metabolism, was made by Magnus and Keizer in pancreatic β-cells (Keizer and Magnus, 1989; Magnus and Keizer, 1997, 1998a,b). Their first model included six transport mechanisms in the inner mitochondrial membrane: proton

pumping via respiration, proton uptake by way of the F_1F_0-ATPase, a proton leak, adenine nucleotide exchange, Ca^{2+} uptake via the MCU, and extrusion via the Na^+/Ca^{2+} exchanger. The kinetic models of each mechanism were developed separately and shown to successfully reproduce the rates of transport measured experimentally. When combined, these mechanisms were used to describe resting mitochondria and phosphorylating mitochondria, by fixing NADH and Ca^{2+} concentrations. Under these conditions, variation of mitochondrial Ca^{2+} concentration was then used to describe mitochondrial Ca^{2+} handling.

Using this minimal model, Magnus and Keizer predicted a very sharp increase in the mitochondrial ability to take up Ca^{2+} at normal cytosolic Ca^{2+} concentrations (i.e. 0.4–0.5 μM), in agreement with experimental observations (Saavedra-Molina et al., 1990). By extending the model to include a more physiological formulation of energy metabolism, which included the Ca^{2+} dependence of mitochondrial dehydrogenases, as well as a dynamic formulation of plasma membrane currents, Magnus and Keizer (1997, 1998a,b) showed that in phosphorylating mitochondria, when NADH levels are constant, the depolarizing influence of Ca^{2+} influx via the MCU would decrease phosphorylation and increase oxidation. Although they predicted this effect to be quite large at cytosolic Ca^{2+} concentrations exceeding 1–2 μM, they showed that this significantly affects ATP production, enough to provoke adverse reactions from ATP-dependent plasma membrane ionic channels, even at lower, more physiological concentrations.

Elements of the Magnus and Keizer models were used by Cortassa et al. (2003) to develop a model of isolated cardiac mitochondria. For better qualitative approximation of the system, extensive modifications, including the addition of NADH as a dynamic state variable dependent upon the activity of the tricarboxylic citric acid (TCA) cycle, were applied. The resulting model provided the first mathematical means of studying the dynamic regulation of energy metabolism by Ca^{2+} cycling in cardiac mitochondria. Model simulations suggested that increases in cytosolic $[Ca^{2+}]$ had two opposite effects on mitochondria: a dissipative effect on the inner mitochondrial membrane due to the shuttling of the divalent cation through the MCU, and a stimulatory effect on the activity of the TCA cycle dehydrogenases resulting from higher intramitochondrial $[Ca^{2+}]$. They concluded that increase in ATP production by Ca^{2+} can only be achieved when the extent of NADH production exceeds the depolarizing effect of Ca^{2+} influx on the change in membrane potential.

By embedding a similar formulation of mitochondria energetics into a whole-cell model of excitation–contraction coupling in the ventricular myocyte, Nguyen and Jafri (2005) were able to study the effects of cytosolic Ca^{2+} transients on Ca^{2+} cycling in the mitochondria, and on energy metabolism. The model predicted that in addition to activation of the TCA cycle dehydrogenases, Ca^{2+}-dependent activation of the F_1F_0-ATPase is necessary in order to achieve significant increases in ATP production. They predicted that mitochondria exposed to the small Ca^{2+} transients in the bulk myoplasm undergo a mild inner membrane depolarization (~10%) in response to such transients, while IMFM located in close proximity to the Ca^{2+} release sites (see Cheng et al., 1993, 1996) undergo depolarization large enough to cause a decline in ATP production that recovers quickly, minimizing its impact on the overall ATP production. The latter provided insight into the self-inhibitory mechanism of mitochondrial Ca^{2+} uptake characterized experimentally as the RaM (Buntinas et al., 2001; Sparagna et al., 1995). This mechanism describes intramitochondrial free $[Ca^{2+}]$ concentration regulation of both Ca^{2+} uptake, and Ca^{2+} extrusion, through changes in membrane potential. The Nguyen and Jafri model also reproduced intramitochondrial Ca^{2+} oscillations in response to pacing, with Ca^{2+} rising to approximately 1/3 of the cytosolic Ca^{2+} concentration (at 1 Hz frequency), in agreement with experimental measurements by Trollinger et al. (2000).

In a more comprehensive model of the ventricular myocyte, Cortassa et al. (2006) integrated all major cellular ATP consuming processes, thereby providing an all-inclusive platform for studying the relationship between cardiac energy supply and demand, and through it, the interplay between Ca^{2+} cycling in the SR and in the mitochondria. Researchers have used this platform to study cellular energetics during physiological and pathophysiological excitation–contraction coupling (Korzeniewski, 2007; Maack and O'Rourke, 2008; Maack et al., 2006; O'Rourke and Maack, 2007; Plank et al., 2008). There have been other notable modeling attempts to link electrophysiology, ion homeostasis, Ca^{2+} handling, ATP consumption, and mitochondrial energetics. One model developed by Matsuoka et al. (2004) used Ca^{2+} as the sole signaling molecule to study the interaction between the cytoplasmic and mitochondrial spaces. Despite being based on sound electrophysiology, the interrelationship between changes in excitation–contraction coupling and bioenergetics was deemed incomplete due to the lack of significant respiratory control in the mitochondrial component of the model (Korzeniewski and Mazat, 1996). Others have elegantly modeled the energetic processes in the mitochondria but do not incorporate the electrophysiological components that regulate cytosolic Ca^{2+} cycling (Saks et al., 2001, 2004; Vendelin et al., 2000, 2004).

Current mathematical models of mitochondrial bioenergetics support intrinsic interactions between SR and mitochondrial Ca^{2+} cycling by way of regulating energy metabolism in the mitochondria. These models are fitted to data obtained from experiments conducted on isolated mitochondria, and validated by their ability to reproduce results obtained *in vitro*. Moreover, the scarcity of experimental data on intact mitochondria, particularly on the spatial geometry of the inner mitochondrial matrix, limits accurate modeling of phenomena such as RaM and spontaneous local depolarization events. For this reason, these models provide only a semiqualitative means of studying bioenergetics in intact mitochondria. In all, the predictive accuracy of mathematical models is restricted by the paucity of detailed spatially and temporally resolved experimental evidence.

5. Future prospects

5.1. Mathematical modeling

The limitations of current mathematical models of mitochondria and how they interact with the cellular and subcellular environment are many. One of the key deficiencies is that the models do not fully include spatially and temporally resolved relationships between Ca^{2+} cycling in the cytosol, in the SR and in the mitochondria. In current models, both SR and mitochondria are each largely modeled as individual homogeneous ("lumped") compartments. Experimental evidence discussed earlier suggests that the ultrastructure of both mitochondria and SR play a critical role in the regulation of Ca^{2+} cycling within these compartments. The conceptual differences in the lumped versus spatially resolved models have not yet been convincingly articulated. Our inability to account for the existence of metabolic compartments (and subcompartments), which is supported by the heterogeneous distribution of ATP within the cell (Saks et al., 1996), was acknowledge by Cortassa et al. (2006) to negatively affect the accuracy of the models. Additionally, Ca^{2+} sparks and the rapid uptake mode (RaM) in mitochondria, which both suggest local intracellular compartments of rapid Ca^{2+} dynamics, are not considered. A starting point in addressing these limitations is a more physiological formulation of SR Ca^{2+} release. This would require inclusion of Ca^{2+} release units (Jafri et al., 1998) that would generate Ca^{2+} sparks (Sobie et al., 2002), such

Table 1
Inhibitors of mitochondrial Ca^{2+} cycling: their direct targets, commonly used concentrations and known effects on the cardiac SR Ca^{2+} cycling.

Inhibitor	Mitochondrial target	IC_{50} (μM)	Concentrations used (μM)	Effect on SR Ca^{2+} cycling	
				Release	Uptake
CGP 37157	mNCX	0.36[10]	1–100[4,5,10,17,19,24,26]	Unknown	No[?10]
Clonazepam	mNCX	7.00[10]	5–100[7,23]	Unknown	No[?10]
Ru360	MCU	0.184[16]	0.1–10[11,19,22,26]	No[?16]	No[?16]
Rotenone	H^+ transport	<1[?14,20]	1–50[4,8,14,27,29]	Unknown	Unknown
Cyclosporin A	PTP	10[?12], <0.1[?25]	0.01–25[1–3,5,9,13,5,21,25–27,29]	No[?9]; Yes[?6,18]	Unknown

MCU, electrogenic mitochondrial Ca^{2+} uniporter; mNCX, mitochondrial Na^+/Ca^{2+} exchanger; NHE, Na^+/H^+ exchanger; nSR, network SR; PTP, permeability transition pore; "?", questionable; 1, Akar et al. (2005); 2, Altschafl et al. (2007); 3, Appaix et al. (2002); 4, Arnaudeau et al. (2001); 5, Ban et al. (1999); 6, Bandyopadhyay et al. (2000); 7, Brandes and Bers (2002); 8, Budd et al. (1997); 9, Budd and Nicholls (1996); 10, Cox et al. (1993); 11, Gincel et al. (2001); 12, Jordani et al. (2000); 13, Kang et al. (2007); 14, Liu et al. (1970); 15, Loupatatzis et al. (2002); 16, Matlib et al. (1998); 17, Pacher et al. (2000); 18, Park et al. (1999); 19, Raiteri et al. (2002); 20, Rowlands and Casida (1998); 21, Salnikov et al. (2007); 22, Sanchez et al. (2001); 23, Sedova and Blatter (2000); 24, Sheu and Sharma (1999); 25, Szabo and Zoratti (1991); 26, Szalai et al. (2000); 27, Trost and Lemasters (1997); 28 Vanden Hoek et al. (1997); 29, Wang et al. (2008).

that the cytosolic Ca^{2+} transient is a summation of these individual release events. A partitioning of the mitochondrial compartment such that some regions are exposed to the larger local $[Ca^{2+}]$ near the jSR end of the IMFM, while other mitochondrial compartments interact with $[Ca^{2+}]$ in the bulk cytoplasm, is also important. The jSR regions may then be the equivalent of microdomains and possible sites of RaM. Although spatially resolved structures and functions of virtually all critical mitochondrial elements are unknown, one may be able to take steps to approach this goal. For example, parameters could be derived through parameter fitting, where known biophysical properties of the system are used as boundary conditions. Such approaches have been successfully employed in mathematical models of excitation-contraction coupling, and to model Ca^{2+} dynamics in the junctional cleft (Cannell et al., 2006; Jafri et al., 1998; Shannon et al., 2004; Soeller and Cannell, 2004). The resulting models would provide a more detailed and physiologically realistic estimate of cardiac cellular energetics and Ca^{2+} signaling with respect to both the mitochondria and SR and their interactions under the modeled conditions.

5.2. Effects of mitochondrial agents on the sarcoplasmic reticulum Ca^{2+} cycling

Table 1 summarizes information about the direct targets of the major mitochondrial Ca^{2+} cycling inhibitors and their concentrations used by different authors *in vivo* and *in vitro,* in different cells. One of the most significant restrictions in studies of IMFM-SR crosstalk is the lack of data on the effects of mitochondrial agents on SR Ca^{2+} cycling mechanisms. The table shows that such effects are either unknown or questionable. Moreover, some data for SR Ca^{2+} release were obtained from changes in global $[Ca^{2+}]$ that do not reveal direct targets because (1) inhibition of RyR2 s can result in an actual increase in $[Ca^{2+}]_{SR}$ due to inhibition of Ca^{2+} leakage through RyR2s and (2) activation of RyR2s can decrease $[Ca^{2+}]_{SR}$ due to increase in Ca^{2+} leakage from the SR (Lukyanenko et al., 1996, 2001b). Effects of some of these inhibitors on cardiac SR Ca^{2+} uptake are not well described because the methods used only permit the authors to evaluate their inhibitory effects on SR Ca^{2+} cycling in general (Cox et al., 1993; Matlib et al., 1998). Therefore, more work is needed to characterize the effects of these mitochondrial agents on the SR.

5.3. Development of novel approaches to measure mitochondrial Ca^{2+} cycling in vivo

Recently a number of novel and powerful approaches were developed and used in cardiac cells that should help to resolve some of the issues noted above. These include: proteomics of Ca^{2+}-sensing proteins in heart mitochondria (Balaban, 2006; Hopper et al., 2006; Taylor et al., 2003); mitochondrial matrix-targeted redox- or Ca^{2+}-sensitive fluorescent proteins and photoactivable GFP (Gerencser and Nicholls, 2008; Gerencser et al., 2008; Karbowski et al., 2004, 2006; Wang et al., 2008), measurement of mitochondrial swelling *in situ* by optimized spatial filtering (Gerencser et al., 2008), and measurement of instantaneous velocity vectors of mitochondrial transport and bioenergetic parameters (Gerencser and Nicholls, 2008). For example, using the latter, it was discovered that hippocampal mitochondria with a higher oxidized thiol redox status have lower membrane potentials and are smaller in size. On average, these mitochondria also have higher motility, which only slightly depended on bioenergetic parameters, but is correlated to the size of the mitochondria. This approach could help to distinguish between the three groups of cardiac mitochondria, including possible interchange in their location within the myocyte.

Recently, we developed a novel approach, confocal monitoring of fluorescence from the mitochondrial intermembrane space loaded with a Ca^{2+} sensitive fluorescent dye (Lukyanenko et al., 2008). This approach could have a great advantage in studying local SR-IMFM Ca^{2+} interplay. It will enable or improve: (1) recording of $[Ca^{2+}]$ in close proximity to IMFM, (2) characterization of the dependence of IMFM Ca^{2+} cycling on Na^+, (3) confirmation or rejection of the functional difference between the three subpopulations of cardiac mitochondria, (4) visualization of the changes in mitochondrial size in conjunction with their effects on the SR Ca^{2+} cycling (Ca^{2+} sparks), and finally, (5) clarification of the role of mitochondrial Ca^{2+} cycling in the regulation of SR Ca^{2+} cycling. Recently, after loading the mitochondrial intermembrane space with fluo-3 pentapotassium salt (fluo-3^{5-}) in isolated cardiac mitochondria, we showed that fluo-3^{5-}: (1) enters the mitochondrial intermembrane space through VDAC, (2) emits a brighter fluorescence signal there than in the surrounding solution due to a higher apparent concentration, and, (3) shows no changes in properties in this mitochondrial low pH subdomain (Lukyanenko et al., 2008). We concluded that fluo-3^{5-} may be used as a probe for cardiac mitochondrial research in its original membrane environment and under quasi-physiological conditions. Preliminary experiments in permeabilized ventricular myocytes support these conclusions, and in addition, suggest that during pathologies involving mitochondrial swelling, mitochondria can produce Ca^{2+} release, which may increase Ca^{2+} leakage from the SR.

6. Summary

Research into the role of mitochondrial Ca^{2+} cycling and its relationship to SR Ca^{2+} cycling is likely to benefit our understanding of Ca^{2+} dynamics in heart. Experimental investigations and parallel mathematical modeling at high spatial and temporal resolution are needed and should enable us to better investigate the molecular physiology in normal hearts and more precisely examine Ca^{2+} and electrical dysfunction in heart disease including arrhythmia and failure.

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