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Ca²⁺ dynamics in the mitochondria - state of the art

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Abstract

The importance of [Ca²⁺] in the mitochondrial matrix, [Ca²⁺]_{mito}, had been proposed by early work of Carafoli and others [1], [2] and [3]. The key suggestion in the 1970s [4] was that regulatory [Ca²⁺]_{mito} played a role in controlling the rate of activation of tricarboxylic acid cycle dehydrogenases, important in the regulation of ATP production by the electron transport chain (ETC) during oxidative phosphorylation. This view is now established [5] and [6] and the key questions currently debated are to what extent do the mitochondria acquire and release Ca²⁺, and what impact do mitochondria have on the dynamic Ca²⁺ signal in the cardiac ventricular myocyte [7]. Although investigations of Ca²⁺ dynamics in mitochondria have been problematic, disparate and inconclusive, they have also been both provocative and exciting. A recent special issue of this journal presented contrasting perspectives on the speed, extent and mechanisms of changes in [Ca²⁺]_{mito}, and how these changes may influence cellular spatio-temporal [Ca²⁺]_i dynamics [8]. An audio discussion is also available online [9]. The uncertain nature of the signaling pathways is noted in Table 1 (see below) which shows mitochondrial proteins and processes that are of current focus and which remain contentious. Each of the “items” listed is largely unsettled, or is a “work in progress”. There may be advocates for opposing positions noted or recent discoveries that must still be tested at multiple levels by diverse laboratories. Currently, the first item, the mitochondrial sodium/calcium exchanger (NCLX) [10], appears the most solid with respect to the molecular identification and physiological function, whereas, the recently described candidates of the mitochondrial Ca²⁺ uniporter (MCU) [11] and [12] still need to be verified and broadly examined by the scientific community.

[Ca²⁺]_i dynamics and mitochondria in the heart: an ideal system for studying Ca²⁺-mitochondrial dynamics

Cardiac ventricular myocytes have the highest volume-fraction of mitochondria of any cell in the body: about 33%. Furthermore, the mitochondria in these myocytes are exposed to high [Ca²⁺]_i with every heartbeat. The intermyofibrillar mitochondria are sandwiched between two Ca²⁺ release units (CRUs), each composed of L-type Ca²⁺ channels (LTCC) in the T-Tubule (TT) and ryanodine receptor (RyR2) clusters in the junctional sarcoplasmic reticulum (jSR) (see Fig. 1). There is one CRU at each end of the intermyofibrillar mitochondria [44]. At the CRU ends of these mitochondria, local [Ca²⁺]_i rises to about 10 μM during a [Ca²⁺]_i transient while the middle of the mitochondria (at the M-line of the sarcomere) experience a local [Ca²⁺]_i as high as about 500 nM [45] and [46]. During an isolated Ca²⁺ spark, nearly the same local high [Ca²⁺]_i is seen by the end of mitochondria

closest to the Ca^{2+} spark. (These estimates of spatially and temporally resolved local $[\text{Ca}^{2+}]_i$ come from a combination of cellular experiments and mathematical modeling -- see Williams et al. 2011 and Sobie et al. 2002 [45] and [46]). The $[\text{Ca}^{2+}]_i$ estimated at the CRU end of a mitochondrion is averaged over a 100 nm region near the active CRU and the measurement is done at the peak of a Ca^{2+} spark. The M-line $[\text{Ca}^{2+}]_i$ is estimated at the same time as the $[\text{Ca}^{2+}]_i$ at the CRU end of the mitochondrion but measured at the middle of the mitochondrion. Despite its name, the voltage dependent anion channel (VDAC) is a large conductance channel that is always open to Ca^{2+} , is permeable to both anions and cations and is significantly regulated [47], [48], [49], [50], [51], [52], [53] and [54]. Importantly the cytosolic $[\text{Ca}^{2+}]_i$ that bathes the outer membrane of the mitochondria, determines the $[\text{Ca}^{2+}]$ in the intermembrane space, and hence the $[\text{Ca}^{2+}]$ experienced by the mitochondrial inner membrane. Due to the local $[\text{Ca}^{2+}]_i$ changes with each heart beat and with Ca^{2+} sparks, the mitochondria experience frequent and important changes that affect the $[\text{Ca}^{2+}]_i$ around the outer mitochondrion membrane and both the intermembrane space and the inner membrane. The microdomains of $[\text{Ca}^{2+}]_i$ around the mitochondria are important, change with time (see Fig.1), and are significantly affected by local Ca^{2+} signaling [55]. It is therefore not surprising that there are many regulatory components in the mitochondria that serve to control the Ca^{2+} flux into and out the matrix and thereby set $[\text{Ca}^{2+}]_{\text{mito}}$ (See Table 1). Because the biology of the system is complex, and our ability to directly resolve Ca^{2+} movements into and out of the mitochondrial matrix is poor, fundamental unanswered questions remain unresolved. What is $[\text{Ca}^{2+}]_{\text{mito}}$? How much does $[\text{Ca}^{2+}]_{\text{mito}}$ change with a Ca^{2+} spark or with a normal $[\text{Ca}^{2+}]_i$ transient? How does the Ca^{2+} influx during a single heartbeat influence mitochondrial metabolic behavior? How does mitochondrial Ca^{2+} flux influence the physiologic $[\text{Ca}^{2+}]_i$ transient? How is mitochondrial oxidative phosphorylation affected by Ca^{2+} movement into the mitochondria and by $[\text{Ca}^{2+}]_{\text{mito}}$? Some of these questions have been raised in the JMCC special issue on mitochondria in 2009. See particularly papers on these topics [7], [8], [38], [39], [56] and [57].

In the present issue of JMCC, Zhou et al. (2011) investigate mitochondrial contributions to $[\text{Ca}^{2+}]_i$ signaling in cardiac myocytes using Ca^{2+} spark activity as a tool to monitor RyR2 activity [58]. Their experiments show a clear correlation between mitochondrial membrane potential (ψ_{mito}) and Ca^{2+} spark frequency. Depolarization of ψ_{mito} increased the frequency of Ca^{2+} sparks above control values. Furthermore, the study provides evidence that reactive oxygen species (ROS) generated by mitochondria can alter RyR2 redox state and function. This paper nicely illustrates the complexity of studying the interactions between mitochondria and Ca^{2+} signaling, showing that mitochondria can influence cytoplasmic Ca^{2+} signaling independent of net uptake or release of Ca^{2+} from mitochondria. While this manuscript seeks to address some of the questions stated above, it also raises others that are equally important to our understanding of contributions of mitochondria to $[\text{Ca}^{2+}]_i$.

- 1) What is the nature of the ROS release pathway? What is the precise role of the inner membrane anion channel (IMAC) in this pathway? What does the mitochondrial permeability transition pore (mPTP) opening do? What opens mPTP versus IMAC? What permeates these pathways?
- 2) What roles are played by IMAC versus mPTP in ψ_{mito} changes and in the movement of Ca^{2+} into or out of the mitochondria?
- 3) What are the conceptual and physiological implications of large mitochondrial Ca^{2+} uptake [40], [42], [59] and [60] versus small Ca^{2+} uptake [38], [39] and [41]? What happens to oxidative phosphorylation? To the $[\text{Ca}^{2+}]_i$ transient?

Complementing the questions raised by Zhou et al (2011) are a series of important but provocative findings that merit discussion (see below). Each of the issues raised in the following paragraphs is related to how Ca^{2+} signaling affects mitochondria or to how mitochondria influence subcellular and cell-wide $[\text{Ca}^{2+}]_i$ dynamics.

1. Some surprising findings in mitochondrial biology and the difficult questions they raise

1.1. Mitofusins

Recent studies have used genetically altered animal investigations of specific mitochondrial proteins that have been found to have an impact on mPTP. For example, in mitofusin 2 (*mfn2*) null mice, the mitochondria appear to be protected from both $[\text{Ca}^{2+}]_{\text{mito}}$ dependent activation of mPTP and from ROS activation of mPTP [61]. This is a surprising finding for three reasons. First earlier work has suggested that this protein was an important factor in the process of mitochondrial fusion and this observation is not clearly related to fusion. Second, this investigation found that the absence of *mfn2* provided metabolic benefit while other studies found the opposite [62]. Third, *mfn2* is found in the outer membrane and it is not yet clear how the outer membrane is involved in mPTP behavior. The past favorite outer membrane protein that was thought to contribute to mPTP behavior was VDAC itself but VDAC null animals had a functional mPTP [63].

1.2. IMAC

Akar et al (2005) have investigated a putative inner membrane anion channel that is reported to be sensitive to chlorodiazepam. While provocative in terms of possible function, IMAC must be identified at the molecular level and investigated more broadly by the scientific community. Like mPTP itself, both molecular identification and characterization are needed [21].

1.3. SR/ER-mitochondrial conduits

The “conduit hypothesis” remains poorly articulated but in one form is possibly the most controversial hypothesis regarding mitochondrial function and Ca^{2+} . Yet, as noted below, it may also approach the trivial. The hypothesis arises from confocal and EM imaging which show possible threads or thin structural links between the SR/ER and mitochondria [35] and [37]. This has led to the speculation that there could be structures that position Ca^{2+} sources very close (nanometers) to a mitochondrion or place possible very small conduits between SR/ER and the matrix of the mitochondria [36], [59], [64] and [65]. While suggestive, there has not been compelling structural information showing a conduction path nor has there been convincing biophysical evidence showing the direct transfer of Ca^{2+} from within the Ca^{2+} source to within the mitochondrion. Some support for intimate touching between the SR/ER membrane and the outer mitochondrial membrane comes from electron micrographs and from FRET imaging between protein structures on the outer membrane of the mitochondria (e.g. mitofusin2) and the SR or ER membranes [66]. When all factors are considered, the SR/ER conduit hypothesis seems to be unlikely in its extreme form for three reasons. 1. The SR/ER Ca^{2+} leak is largely accounted for quantitatively [45]. 2. The mitochondria have only small “influx” leak pathways (e.g. the MCU) that appears adequate to provide signaling Ca^{2+} for mitochondrial regulatory needs but not mass quantities [11] and [12]. 3. No utility/need has yet been demonstrated for the mitochondria to be directly loaded with Ca^{2+} from the SR/ER. Nevertheless, the provocative hypothesis may in fact be quite mundane but still important.

The experimental results and structural findings may, however, be consistent with the kind of normal Ca^{2+} signaling seen in heart, as shown in Fig. 1. Here, some regions of the

mitochondria are quite close (nanometers) to the jSR and exposed to about 10 μM $[\text{Ca}^{2+}]_i$ with each local Ca^{2+} spark or $[\text{Ca}^{2+}]_i$ transient. This exposure is a simple result of the local signaling that controls cardiac excitation-contraction coupling and does not depend on “special” conduits into the mitochondria. If the actual “conduit hypothesis” becomes simply a local high $[\text{Ca}^{2+}]_i$ hypothesis, then it depends simply on Ca^{2+} sparks and the local release of Ca^{2+} near the mitochondria [67], [68] and [69]. Such local signaling of mitochondrial ROS to the CRU as reported by Zhou et al (2011) [58] reflects by reciprocity the same local signaling system for Ca^{2+} from the CRU for the mitochondria. This exciting possibility raises again the benefit of using cardiac ventricular myocytes as a model system for studying mitochondrial Ca^{2+} dynamics and related signals.

1.4. Opening of the mitochondrial “permeability transition” pore (mPTP)

The dramatic depolarization of mPTP has often been viewed as a catastrophic event for the mitochondrion, one that leads to release of intermembrane cytochrome C and the initiation of apoptosis [70] and [71]. Nevertheless, there have been speculations about mPTP “flickers” or brief and reversible openings that may be physiologically important [72], [73], [74] and [75]. More specific molecular information on mPTP may be needed to investigate these matters. While the biophysical and molecular nature of the mPTP remains elusive, data to date suggests that elevated $[\text{Ca}^{2+}]_{\text{mito}}$ or elevated ROS_{mito} (or both) appears to contribute to the opening of the mPTP. When the mPTP opens, large molecules that may be present in the cytosol (like the fluorescent molecule calcein, MW = 623) can enter the mitochondrial matrix from the cytosol and thus be used to monitor the opening of mPTP. Thus as the mPTPs in an individual mitochondrion open, the mitochondrion depolarizes and both large and small molecules like Ca^{2+} and ROS (e.g. H_2O_2 or superoxide) can freely pass into and out of the mitochondrial matrix passing into the intermembrane space and cytosol [21], [57] and [75]. From a Ca^{2+} centric position we must still ponder what the effects of mPTP flickers and longer-term openings are on both the kinetics and extent of changes in $[\text{Ca}^{2+}]_{\text{mito}}$ and subcellular $[\text{Ca}^{2+}]_i$.

1.5. Uncoupling proteins

There are five “uncoupling proteins” (see Table 1) that reside in the inner membrane and shunt protons from the intermembrane space back into the matrix. Candidate proteins are now under investigation [34]. In heart UCP2 is an isoform that has been studied [76] and [77] among others. These UCP's can be important in health and disease [78], and may contribute to mitochondrial adaptation and/or contribution to diabetes and heart failure. It appears that Ca^{2+} plays an important modulatory role in UCP regulation [31], [33] and [79] as do fatty acids [32]. Nevertheless, exactly how Ca^{2+} contributes to UCP regulation remains an area of active study.

1.6. Summary

Exciting recent findings on mitochondrial Ca^{2+} signaling, including the new report in this issue of JMCC [58], suggest that we are rapidly learning about the molecular details of the control of $[\text{Ca}^{2+}]_{\text{mito}}$ as a topic unto itself and also how this signaling affects local and global $[\text{Ca}^{2+}]_i$. It is clear, however, that there is much room for future discovery.

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Fig. 1.

Dynamic $[Ca^{2+}]_i$ signals bathe mitochondria. At the peak of a Ca^{2+} spark, the Z-disk end of an intermyofibrillar mitochondrion will be bathed by $[Ca^{2+}]_i$ at about $10\ \mu M$ while the $[Ca^{2+}]_i$ at the mid-mitochondrion is about $500\ nM$. Data replotted from Williams et al 2011 and Sobie et al 2002 [45] and [46]; see text for more details.

Table 1

Contentious and Developing Components of Cardiac Mitochondrial Calcium Signaling.

Item	Status	Comment	Reference
Mitochondrial sodium-calcium exchanger (NCLX)	Working candidate	NCLX, the mitochondrial sodium/calcium lithium exchanger	[10]
Mitochondrial calcium uniporter (MCU)	Working candidate(s)	Two groups have contributed to discoveries in June 2011.	[11] and [12]
Mitochondrial KATP channel	Molecular identity unknown.	Cardioprotective function against ischemic injury is well established	[13], [14] and [15]
Mitochondrial permeability transition pore (mPTP)	Molecular identity unknown.	Many past candidate components for mPTP have included: VDAC, ANT, cyclophilin D	[16], [17], [18] and [19]
Mitochondrial sodium-hydrogen exchanger	Molecular identity unknown.	Main [Na ⁺]mito efflux pathway. Many unanswered questions remain	[20]
Inner membrane anion channel (IMAC)	Molecular identity unknown.	Proposed as alternate mitochondrial ROS efflux pathway; blocked by high concentrations of chlorodiazepam	[21]
Mitochondrial Ca ²⁺ -activated K channel	Molecular identity unknown.	MitoKca is believed to be cardioprotective against ischemic injury but function under normal conditions is known	[22]
Mitochondrial K ⁺ /H ⁺ exchanger	Molecular identity unknown.	[K ⁺]mito efflux pathway	[20]
Water channels	Working candidate	Aquaporins are permeable to H ₂ O and H ₂ O ₂ ; important in osmotic pressure regulation and in mitochondrial ROS efflux	[23], [24], [25], [26] and [27]
[Proton] in intermembrane space	Spatial gradients within mitochondria not yet tested experimentally	Important in chemo-osmotic hypothesis	[28], [29] and [30]
Uncoupling Proteins (UCP1, UCP2, UCP3, UCP4, UCP5)	Candidate genes have been identified.	inner membrane proton channel, regulated by Ca ²⁺ (and other factors - e.g. fatty acids)	[31], [32], [33] and [34]
ER/SR calcium conduit	Molecular identity unknown	Structural speculation; No physiologic need; no function demonstrated	[35], [36] and [37]
[Ca ²⁺] _{mito}	Evidence for and against transient changes during normal E-C coupling.	All mitochondrial Ca ²⁺ sensors are problematic; Rhod2 is likely to be in both the intermembrane space and matrix; the exact location of the genetically targeted Ca ²⁺ sensors remains uncertain.	[38], [39], [40], [41] and [42]
Other K channels	Molecular identity unknown	Kv1.3 has been reported in lymphocyte mitochondria	[43]