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The Mitochondrial Calcium Uniporter (MCU): Molecular Identity and Physiological Roles*

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Maria Patron, Anna Raffaello, Veronica Granatiero, Anna Tosatto, Giulia Merli, Diego De Stefani, Lauren Wright, Giorgia Pallafacchina, Anna Terrin, Cristina Mammucari, and Rosario Rizzuto[1]

From the Department of Biomedical Sciences, University of Padua and the Institute of Neuroscience, Consiglio Nazionale delle Ricerche, 35131 Padua, Italy

The direct measurement of mitochondrial $[Ca^{2+}]$ with highly specific probes demonstrated that major swings in organellar $[Ca^{2+}]$ parallel the changes occurring in the cytosol and regulate processes as diverse as aerobic metabolism and cell death by necrosis and apoptosis. Despite great biological relevance, insight was limited by the complete lack of molecular understanding. The situation has changed, and new perspectives have emerged following the very recent identification of the mitochondrial Ca^{2+} uniporter, the channel allowing rapid Ca^{2+} accumulation across the inner mitochondrial membrane.

The Complexity of Calcium Signaling

The initial concept that Ca^{2+} ions control physiological events goes back to the seminal observation by Ringer in 1883 that addition of Ca^{2+} to the perfusion buffer of isolated hearts triggered their contraction (1). The development of techniques to monitor changes in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$) (2, 3) allowed the concept that Ca^{2+} ions act as second messengers to be to extended to virtually all cells. The advent of powerful probes allowing single cell analysis (the intracellularly trappable fluorescent indicators (4, 5)) revealed that Ca^{2+} increases can be highly localized (*e.g.* the synaptic region or the secretory pole of an exocrine cell) or diffused across the cell as a Ca^{2+} wave and elicit an effect at a distant site (*e.g.* activate gene transcription in the nucleus of the cell). Moreover, in most cell types, the $[Ca^{2+}]_c$ increases are oscillatory (6), and the frequency of oscillation is differentially decoded by the cell (7, 8). It is beyond the scope of this minireview to discuss the molecular and cellular basis of this spatiotemporal complexity. It suffices to mention two key requirements. The first is the cooperation of different sources of Ca^{2+} in the generation of the $[Ca^{2+}]$ rise: the extracellular medium, a virtually unlimited reservoir with a $[Ca^{2+}]$ of ~1 mM, and intracellular pools, generally referred to as Ca^{2+} stores, which are endowed with a $[Ca^{2+}]$ (>100 μM) that allows rapid release through resident channels (9, 10). Although recent work has highlighted a role also for other membrane-bound compartments (*e.g.* the Golgi apparatus (11) and endo/lysosomes (12)), the most important intracellular stores are the endoplasmic reticulum (ER)[2] and its specialized counterpart of muscle cells, the sarcoplasmic reticulum (SR). The second requirement for such a carefully orchestrated signal is the existence of a broad number of molecules generating and decoding $[Ca^{2+}]$ variations and their defined positioning within the cell (13). Thus, specific pumps, channels, and buffering proteins finely tune the spatiotemporal pattern of the $[Ca^{2+}]_c$ rises, and specific targets, located in both the cytoplasm and different intracellular organelles, are specifically affected by the ionic change.

The Contribution of Mitochondria to Cellular Calcium Handling

Mitochondrial Ca^{2+} Uptake: A Historical Perspective

Mitochondria were the first intracellular organelles to be associated with calcium handling. Indeed, Ca^{2+} uptake by energized isolated mitochondria was directly measured half a century ago (14–16). It is remarkable that this experimental observation anticipated the chemiosmotic theory that provides the thermodynamic basis for rapid accumulation of a positively charged ion into the mitochondrial matrix (17). In the following 2 decades, the process of mitochondrial Ca^{2+} accumulation was thoroughly investigated. Although the outer membrane is known to be permeable to ions and small solutes, transfer across the inner membrane requires the presence of specific transporters. Accordingly, a rapid electrogenic pathway (denoted the "mitochondrial calcium uniporter" (MCU)) was described that rapidly transports Ca^{2+} into the matrix, driven by the negative charge of the membrane potential ($\Delta\Psi$) established by the respiratory chain. Ca^{2+} accumulation partially dissipates the electrochemical gradient, closely agreeing with the early observation that Ca^{2+} stimulates respiration transiently, in contrast with the classical mitochondrial uncouplers. Ca^{2+} accumulation by MCU does not proceed until electrochemical equilibrium (which would lead to a 10^6 concentration difference) due to the activity of two main efflux pathways, the Na^+/Ca^{2+} (mNCX) and H^+/Ca^{2+} (mHCX) exchangers, expressed mainly in excitable and non-excitable tissues, respectively. The existence of a sophisticated machinery for Ca^{2+} handling supported the general consensus that mitochondria could actively and rapidly change their $[Ca^{2+}]$ and participate in cellular homeostasis.

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[1] To whom correspondence should be addressed. E-mail: rosario.rizzuto@unipd.it.

[2] The abbreviations used are: ER, endoplasmic reticulum; SR, sarcoplasmic reticulum; MCU, mitochondrial calcium uniporter; mNCX, mitochondrial Na^+/Ca^{2+} exchanger; mHCX, mitochondrial H^+/Ca^{2+} exchanger; IP_3, inositol 1,4,5-trisphosphate; RyR, ryanodine receptor; IP_3R, IP_3 receptor; PTP, permeability transition pore; OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; RuR, ruthenium red; TM, transmembrane.

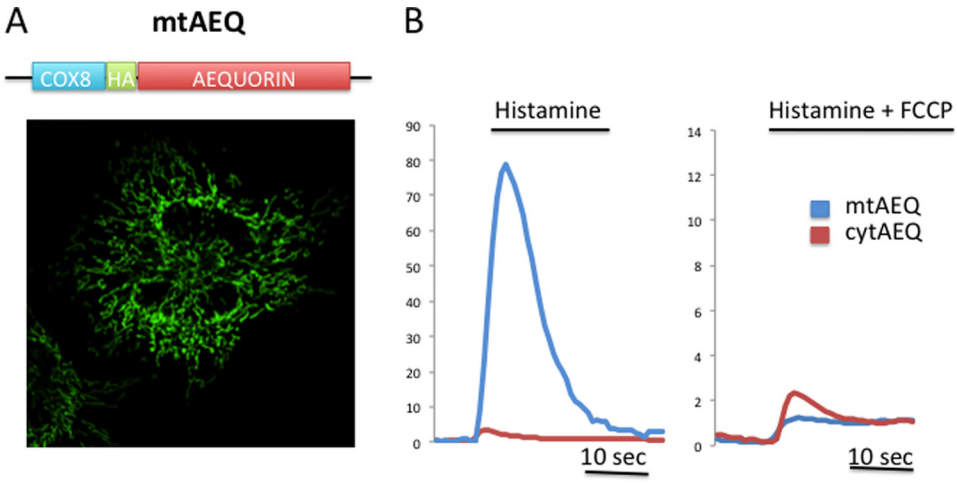

FIGURE 1. *A*, schematic representation (*upper*) and immunolocalization (*lower*) of the mitochondrial aequorin (*mtAEQ*) probe (showing correct targeting to mitochondria); *HA*, haemagglutinin epitope. *B*, $[Ca^{2+}]_m$ (*blue traces*) and $[Ca^{2+}]_c$ (*red traces*) measurements in HeLa cells upon treatment with 100 μM histamine in the absence (*left panel*) and presence (*right panel*) of the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (*FCCP*). *cytAEQ*, cytosolic aequorin.

However, this idea was strongly questioned in the 1980s by two key observations. The first was the demonstration by Berridge and co-workers (18) that inositol 1,4,5-trisphosphate (IP_3), generated upon stimulation of receptors coupled to phospholipase C, mobilizes Ca^{2+} from a "non-mitochondrial" Ca^{2+} store, the ER. Furthermore, another seminal study described the relative affinities for calcium uptake by mitochondria and the ER (19). Quite appropriately, the sarco/endoplasmic reticulum became the main research focus in calcium signaling, leading to the identification of resident molecules involved in Ca^{2+} transport, the IP_3- and ryanodine receptor (RyR)-sensitive channels, and the sarco/endoplasmic reticulum ATPase (10). The second key observation was the accurate $[Ca^{2+}]_c$ measurement obtained, in virtually all cell types, with fluorescent indicators. It became clear that given the $[Ca^{2+}]_c$ measured under resting (~0.1 μM) or stimulated (1–3 μM at the peak) conditions, MCU would not allow substantial Ca^{2+} uptake into the organelle. The general consensus thus changed, and mitochondria gradually faded away from the general picture of cellular Ca^{2+} homeostasis, as significant Ca^{2+} uptake was predicted to occur only under conditions of massive Ca^{2+} overload.

The Resurrection of Mitochondria and the Microdomain Concept

The pendulum swung again in the early 1990s. Although mitochondrial Ca^{2+} uptake had already been described (20), the development of recombinant targeted probes was crucial for the reappraisal of the role of mitochondria. The pioneer of these probes was the photoprotein aequorin (21), which was followed by GFP-based fluorescent probes allowing the direct visualization of $[Ca^{2+}]$ changes in imaging experiments (4, 22). These highly specific probes clearly revealed that when a cell is stimulated with a $[Ca^{2+}]_c$-increasing agonist, mitochondria always accumulate Ca^{2+} in the matrix with a speed and an amplitude that greatly exceed those expected from the properties of MCU in isolated mitochondria (23). Indeed, the upstroke of the mitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_m$) rise closely follows that of the cells (24), and the peak value reaches, in some cell types, values in excess of 100 μM (Fig. 1) (25).

The apparent discrepancy with the low affinity of MCU was solved by the demonstration that mitochondria are located in close proximity to the Ca^{2+} channels eliciting the Ca^{2+} rise, *i.e.* the IP_3 receptors (IP_3Rs; or RyRs) in the ER/SR (26–29), or different classes of channels on the plasma membrane (*e.g.* voltage- and store-operated channels) (30–33). Therefore, they sense a microdomain of high $[Ca^{2+}]$ that meets the low affinity of MCU and is then dissipated, thus preventing mitochondrial Ca^{2+} overload and/or vicious Ca^{2+} cycling across the mitochondrial membrane. This idea was initially supported by the morphological demonstration of the close proximity of the two organelles and by the evidence that only perfusion of IP_3 or of Ca^{2+} at a 10-fold higher concentration than that measured in the bulk cytosol could induce rapid Ca^{2+} uptake in permeabilized cells (23). More recently, the $[Ca^{2+}]$ reached on the outer surface of mitochondria was directly measured, thus providing reliable estimates of the $[Ca^{2+}]$ microdomain sensed by mitochondria (34, 35). The concept that mitochondria respond to a pulsatile event, the generation of a rapidly dissipating microdomain, has major functional implications. The first stems from the old observation that both ATP phosphorylation and Ca^{2+} uptake occur at the expense of mitochondrial $\Delta\mu_{H^+}$. During cell stimulation, continuous accumulation (and cycling) of Ca^{2+} across the mitochondrial membrane would interfere with ATP production, whereas a rapid and transient response allows a functional response with limited energy drain, as discussed below. The second crucial implication of a microdomain-based signaling mechanism is that the shape and positioning of mitochondria within the cell become critical determinants of their responsiveness to Ca^{2+} signals. Thus, not only in neurons, where localization in synaptic regions was known to underlie rapid Ca^{2+} uptake (and Ca^{2+} buffering), but in most cells, mitochondria appear to be docked to dedicated signaling sites known as the mitochondria-associated ER membrane (36). In addition, the highly regulated state of fusion/fission influences the mitochondrial Ca^{2+} responses, as fragmentation discon-

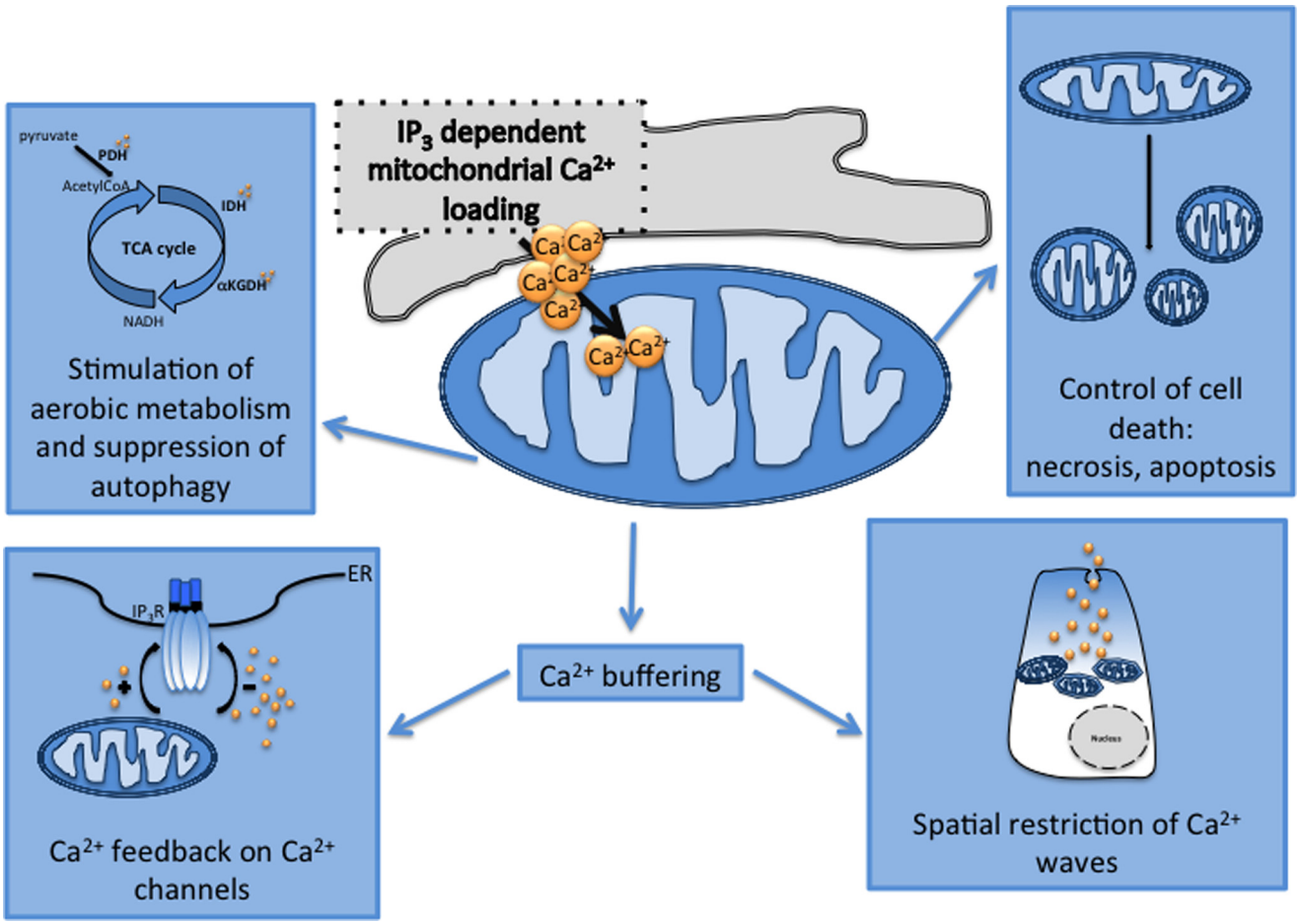

FIGURE 2. **Pleiotropic roles of mitochondrial Ca^{2+} homeostasis.** *PDH*, pyruvate dehydrogenase; *IDH*, isocitrate dehydrogenase; *αKGDH*, α-ketoglutarate dehydrogenase.

nects part of the mitochondria from the signaling sites (37). MFN2 (mitofusin 2), a critical component of the mitochondrial fusion/fission machinery, is enriched in mitochondria-associated ER membranes, where it was shown to tether the ER and mitochondria (38), thus participating in the formation of mitochondria-ER contacts. Indeed, in fibroblasts lacking MFN2, the distance between the ER and mitochondria increases, and mitochondrial Ca^{2+} uptake is drastically reduced (38).

The Physio(patho)logical Role of Mitochondrial Calcium Handling

The demonstration that mitochondria rapidly accumulate Ca^{2+} upon stimulation was followed by a large body of work that allowed the physiological (and pathological) role of mitochondrial Ca^{2+} homeostasis to be established in the following years (Fig. 2). At first, the role in the control of aerobic metabolism was directly investigated. Such a role was predicted based on the biochemical evidence, dating back to the 1970s, that three matrix dehydrogenases are activated by Ca^{2+}: pyruvate dehydrogenase is regulated by a Ca^{2+}-dependent phosphatase, and α-ketoglutarate and isocitrate dehydrogenases is regulated by direct binding of Ca^{2+} to the enzyme (39, 40). Stimulation of Ca^{2+}-sensitive dehydrogenases increases NADH availability and hence the flow of electrons down the respiratory chain, thus, in principle, enhancing a rate-limiting step for rapid ATP synthesis in stimulated cells.

Increased electron feeding into the respiratory chain allows an increase in ATP production, as demonstrated by the direct measurement of ATP levels in the mitochondria and cytosol. Interestingly, this Ca^{2+}-mediated activation of aerobic metabolism appears to be impaired in some mitochondrial genetic disorders, such as respiratory chain defects, in which the partial reduction of $\Delta\Psi$ greatly impairs the transmission of Ca^{2+} signals to mitochondria and hence ATP production. Accordingly, in cell lines harboring these mutations (such as the tRNA defects), restoration of Ca^{2+} signals (*e.g.* using inhibitors of mitochondrial Ca^{2+} efflux) markedly increases ATP production (41).

Importantly, mitochondrial Ca^{2+} is also involved in the regulation of cell death pathways. Mitochondrial Ca^{2+} overload has long been known to be associated with the process of necrosis, such as that due to ischemia-reperfusion of the heart and excitotoxicity of neurons (for a review, see Ref. 42). Cellular (and hence mitochondrial) Ca^{2+} overload, in conjunction with accumulation of reactive oxygen species, favors the sustained opening of the high conductance cyclosporin A-sensitive permeability transition pore (PTP), which causes the rapid collapse of $\Delta\Psi_m$ and the swelling of mitochondria, with consequent loss of pyridine nucleotides and cytochrome *c*. The ensuing bioenergetic crisis and ATP depletion send the cells to necrotic cell death (43, 44).

In more recent years, however, Ca^{2+} dysregulation and, more specifically, $[Ca^{2+}]_m$ increases have been shown to play a regulatory role in the more controlled process of apoptotic cell death. The initial evidence was provided by the demonstration that the anti-apoptotic oncogene *bcl-2* affects intracellular Ca^{2+} homeostasis by regulating the Ca^{2+} leak from the ER and/or the release kinetics upon cell stimulation. The most logical target for this signaling alteration was mitochondria, which, in the initiation step of the intrinsic pathway of apoptosis, release essential components of the apoptosome (such as cytochrome *c*, SMAC/DIABLO, and apoptosis-inducing factor). In this release, a key role is played by organelle fragmentation and swelling following PTP opening (45), a process triggered by increases in matrix $[Ca^{2+}]$, in conjunction with a variety of pathological challenges (*e.g.* oxidative stress or production of C_2-ceramide). Thus, in a stressed cell, cytosolic Ca^{2+} waves, such as those evoked by a physiological stimulation, rather than stimulating aerobic metabolism, trigger an intracellular wave of PTP opening that ultimately leads to cell death. Interestingly, in the ER, Bcl-2 reduces mitochondrial Ca^{2+} loading (46–49), whereas the pro-apoptotic members of the protein family (such as Bax) exert the opposite effect (50). Furthermore, Bcl-2 localizes also to mitochondrial membranes, where it has been demonstrated to protect cells against mitochondrial Ca^{2+} overload (51).

Mitochondrial Ca^{2+} signals have recently been shown to play a major role in the regulation of autophagy. Indeed, a recent seminal paper by Foskett and co-workers (52) clearly demonstrated that $[Ca^{2+}]_m$ exerts an inhibitory effect on AMP-activated protein kinase activity. Indeed, the authors showed that in DT40 $IP_3R1/2/3$ knock-out cells (in which IP_3R-dependent cellular Ca^{2+} signals were abrogated), autophagy was maximally activated also in the presence of nutrients (52). The effect could be ascribed to ablation of mitochondrial Ca^{2+} loading, as MCU blockers had the same effect as IP_3R inhibition. This study led to the interesting conclusion that apoptosis and autophagy, which share molecular regulators (such as Beclin), also utilize a common second messenger (Ca^{2+} and its loading into mitochondria) to drive cell fate in opposite directions (high mitochondrial $[Ca^{2+}]$, pro-cell death; low $[Ca^{2+}]$, autophagic rescue).

Finally, the consequences of mitochondrial accumulation are not limited to events occurring within the organelle. Indeed, the fact that mitochondria can take up large Ca^{2+} loads (albeit with a low affinity mechanism) places them, at least in principle, among the buffers that should be taken into account when estimating the factors that reduce the amplitude of the cytosolic $[Ca^{2+}]$ increases evoked by channel opening and/or that slow the diffusion of Ca^{2+} waves. Although we refer to more exhaustive reviews for a detailed coverage of this topic, we will briefly sketch two conceptually different mechanisms through which mitochondrial buffering plays a functional role (53).

The first relates to the possibility that strategic positioning of mitochondria endows cells with a highly polarized complex morphology with a high capacity sink draining a large amount of Ca^{2+} and preventing (or delaying) Ca^{2+} diffusion to distant sites. In pancreatic acinar cells, a mitochondrial belt maintains Ca^{2+} rises in the apical region (where they are generated), limiting its effect to granule secretion. When the buffering capacity of mitochondria is overwhelmed, as in supramaximal stimulation or by impairing mitochondrial uptake capacity, the Ca^{2+} wave diffuses to the whole cell, thus reaching also the nucleus, where it promotes gene transcription and long-lasting changes (54). In neurons, mitochondria located in proximity to the plasma membrane accumulate large amounts of Ca^{2+} (up to >10 mmol/kg of dry weight), most likely stored as calcium phosphate precipitates (55). By this means, they modulate synaptic $[Ca^{2+}]$ increases, strongly affecting neurotransmitter release. Interestingly, genetic ablation of a low affinity Ca^{2+}-binding protein highly expressed in neurons (parvalbumin) induces mitochondrial biogenesis and redistribution as a compensatory mechanism, thus indicating that the cell regulates these organelles as key components of its Ca^{2+}-buffering repertoire (56).

The second, conceptual mechanism is the generation of signaling microdomains with the ER/SR or the plasma membrane, in which mitochondrial Ca^{2+} uptake controls the microenvironment of the resident channels, thus modulating the positive or negative feedback of the cation on the channel itself. A revealing example was provided in the late 1990s by Lechleiter and co-workers (57), who demonstrated that Ca^{2+} uptake by energized mitochondria controls the kinetics of IP_3R Ca^{2+} release and hence coordinates IP_3-induced Ca^{2+} waves. Interestingly, this concept is not restricted to ER channels but plays a role also in the control of plasma membrane channels. A large body of work has been carried out in T lymphocytes, which depend on store-operated channel activity to sustain the prolonged $[Ca^{2+}]$ increases needed for cell activation (58). In this system, mitochondria buffer the local $[Ca^{2+}]$ increases in the proximity of the channel, thus preventing rapid inactivation. Interestingly, in mast cells, mitochondria are more distant from the plasma membrane but still participate in shaping the $[Ca^{2+}]$ increase and hence the functional response by buffering the global cytosolic rise, thus influencing both slow inactivation of ORAI channels and ER Ca^{2+} loading (30).

The Essential Components of the Ca^{2+} Transport Machinery

Outer Mitochondrial Membrane

Ca^{2+} accumulation in the mitochondrial matrix requires the crossing of two membranes, the outer (OMM) and inner (IMM) mitochondrial membranes. Attention has been placed mostly on the transport systems of the ion-impermeable IMM, as the OMM is permeable to solutes of <5 kDa and thus also to Ca^{2+}. However, the dependence of rapid Ca^{2+} uptake into the matrix on high $[Ca^{2+}]$ microdomains generated at the mouths of open ER/SR (or plasma membrane) channels implied that also passive equilibration across the OMM could represent a rate-limiting step. The high permeability of the OMM is due mostly to its most abundant protein, the voltage-dependent anion channel, which allows the shuttling of all energy-related metabolites, including succinate, pyruvate, malate, NADH, ATP, ADP, and phosphate, from the cytosol to the mitochondria. Voltage-dependent anion channel overexpression augments agonist-dependent Ca^{2+} rises, implying that the repertoire of OMM chan-

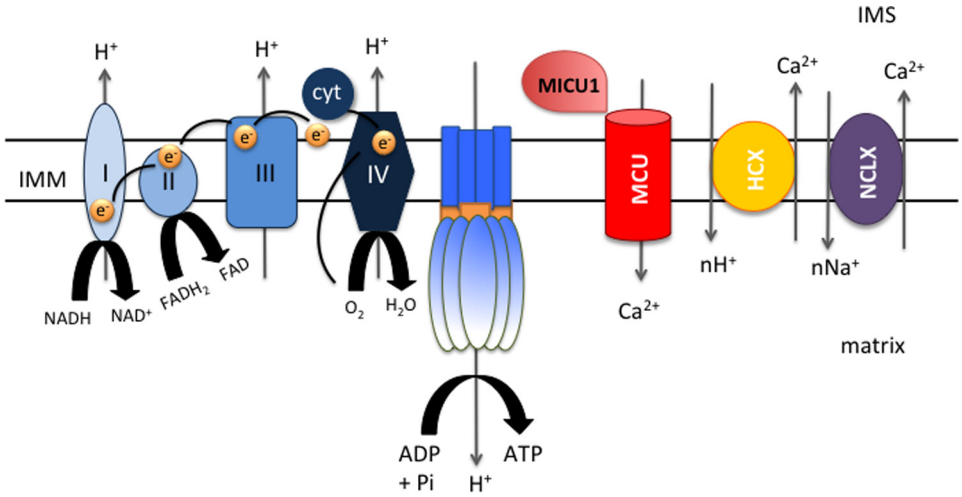

FIGURE 3. **Schematic representation of the essential molecular components of mitochondrial Ca^{2+} homeostasis: the electron transport chain complex, building up the electrical driving force for accumulation ($\Delta\Psi$), MCU, and the mNCX and mHCX exchangers.** *cyt*, cytochrome *c*; *IMS*, intermembrane space.

nels is a kinetic bottleneck in the transfer of the Ca^{2+} microdomain to the IMM (59, 60).

Inner Mitochondrial Membrane

Thermodynamic Principles—A much more substantial problem for Ca^{2+} accumulation in the matrix is represented by the ion-impermeable inner membrane (Fig. 3). Indeed, the electron transport chain translocates H^+ ions across the IMM, generating an electrochemical proton gradient ($\Delta\mu_{H^+}$), which is mostly in the form of a membrane potential difference ($\Delta\Psi$). $\Delta\mu_{H^+}$ drives the flow of H^+ through the ATP synthase in a reaction coupled to the generation of ATP from ADP and inorganic phosphate (61) or through leak pathways, such as the uncoupling proteins (which cause H^+ cycling and increased respiration and heat production) (62). $\Delta\Psi$ also represents a very large driving force for Ca^{2+} accumulation. Based on the Nernst equation (Equation 1, where cat is cation),

$$V_{eq} = \frac{RT}{zF} \ln\left(\frac{[cat^{z+}]_{out}}{[cat^{z+}]_{in}}\right) \qquad \text{(Eq. 1)}$$

equilibrium is reached at an inside $[Ca^{2+}]$ 10^6-fold greater than that outside the IMM.

The Transporters—Ca^{2+} influx occurs via an electrophoretic pathway called the MCU. Ca^{2+} accumulation by MCU is counteracted predominantly by mNCX (63). This exchanger, first described by Carafoli and co-workers (64), was recently identified by Sekler and co-workers (65), who demonstrated that silencing of NCLX blocks Ca^{2+} extrusion and that NCLX-mediated mitochondrial Ca^{2+} transport is inhibited by the NCX blocker CGP-37157.

Both exchangers are ruthenium red (RuR)-insensitive, and indeed, in classical experiments in isolated mitochondria, after challenging mitochondria with a Ca^{2+} pulse, exchanger-mediated efflux was initiated by RuR addition. mNCX is inhibited by a group of benzothiazepine analogs, such as diltiazem, clonazepam, and CGP-37157 (66). Its V_{max} ranges between 0.6 (liver) and 18 nmol (heart) of Ca^{2+}/mg of protein/min (67).

The Discovery of the MCU: Finding the Needle in the Mitochondrial Haystack

Through the years, there has been a wide consensus that the key molecule allowing rapid accumulation of Ca^{2+} across the ion-impermeable inner channel is the electrophoretic pathway called MCU. Studies in isolated mitochondria demonstrated that it is inhibited by RuR (68) and lanthanides (69), with a V_{max} of >1400 nmol of Ca^{2+}/mg of protein/min (70). Electrophysiological recordings of isolated mitoplasts demonstrated that MCU is a highly selective, inwardly rectifying Ca^{2+} channel (71, 72).

Several candidates have been proposed through the years until the recent discovery. After a few tentative identifications of putative RuR-binding glycoproteins in the 1970s (73), in a series of papers, Sheu and co-workers (74, 75) proposed that mitochondrially sorted RyR1 drives mitochondrial Ca^{2+} uptake in the heart. Although the tissue distribution and electrophysiological properties seem to exclude RyR1 as the MCU, the possibility that RyR is an additional uptake pathway cooperating with MCU in the heart is still open. More recently, Graier and co-workers (76) proposed that UCP2 and UCP3 are essential components of the MCU machinery. These results, which were questioned on theoretical grounds and based on experiments carried out in mitochondria from $UCP2^{-/-}$ and $UCP3^{-/-}$ mice (77), have recently been ascribed to an indirect effect on ATP production and hence ER Ca^{2+} loading (78). Finally, in 2009, Clapham and co-workers (79) identified, by siRNA genomic screening in *Drosophila*, Letm1 as a putative H^+/Ca^{2+} antiporter mediating Ca^{2+} accumulation in the matrix. Given that the same protein was proposed to be a mitochondrial K^+/H^+ exchanger (80) and that the proposed stoichiometry and RuR sensitivity differ from those reported for mNCX and mHCX (81, 82), the role of Letm1 awaits further confirmation and, in all cases, does not reflect MCU activity.

The success in MCU identification required an innovative approach. Indeed, (i) the available inhibitors of channel function (*e.g.* RuR) lacked the protein specificity necessary for direct

protein identification; (ii) *Saccharomyces cerevisiae* appears to have no RuR-sensitive mitochondrial Ca^{2+} influx (83), thus preventing the use of yeast genetics; and (iii) *in silico* homology searches with known Ca^{2+} channels yielded no successful hit. An alternative strategy was made possible by the seminal work of Mootha and co-workers (84), who constructed a mitochondrial gene data set (MitoCarta) by compiling an inventory of gene products with proven mitochondrial localization. By searching the MitoCarta data set, the same group identified a 54-kDa protein, renamed MICU1 (mitochondrial calcium uptake 1). Its silencing in HeLa cells drastically reduced mitochondrial Ca^{2+} uptake, thus proving that it is an essential component of the Ca^{2+} uptake machinery (85). However, MICU1 includes two Ca^{2+}-binding EF domains and a single putative transmembrane (TM) domain and thus was unlikely to be the channel itself.

The presence of an MCU regulator further stimulated the search for MCU, and indeed, two groups (our own (87) and Mootha's (88)) found MCU in 2011 through an *in silico* search of the MitoCarta database. Specifically, we skimmed the list by applying subsequent constraints: 1) a broad expression profile, given the presence of RuR-sensitive mitochondrial Ca^{2+} uptake in all mammalian tissues; 2) at least two computationally predicted TM α-helixes in the primary sequence (*i.e.* the expected minimum requirement of all ion channels); 3) the absence in *S. cerevisiae*, which lacks RuR-sensitive mitochondrial Ca^{2+} uptake; and 4) the presence in Trypanosomatida, in which RuR-sensitive Ca^{2+} uptake was reported (86). We ended up with a list of 14 candidates, containing both well known (*e.g.* subunits of NADH dehydrogenase) and yet unexplored proteins. We finally reasoned that given the key function of ion permeation, the evolutionary pressure was most likely focused on conserving the TM domains and the selectivity filter rather than other traits. We thus carefully aligned the 14 TM domains and intervening loops of candidates across distant species, and it was immediately clear that one of the hits (the product of the *CCDC109A* gene) met our predictions: two very highly conserved TM domains separated by a small loop identical in sequence from human to kinetoplastids and enriched in acidic residues. *CCDC109A* (then renamed MCU) became the leading candidate and was validated in studies in intact and permeabilized cells (Fig. 4). Interestingly, Mootha and co-workers (87) also identified the same candidate using a different computational approach. They searched for genes closely related to *MICU1* in terms of evolutionary co-occurrence and mRNA/protein coexpression profiles, identifying *CCDC109A* as the top scoring hit.

MCU silencing virtually abolishes mitochondrial Ca^{2+} uptake in intact and permeabilized cells (87, 88). We cloned the MCU cDNA and demonstrated that its overexpression doubled the $[Ca^{2+}]_m$ rise evoked by IP_3-coupled agonists while significantly reducing the amplitude of the $[Ca^{2+}]_c$ peaks as a consequence of the increased mitochondrial Ca^{2+} buffering (87). These data demonstrated a crucial role also of this protein in mitochondrial Ca^{2+} uptake, but direct evidence was necessary to claim that it is MCU, *i.e.* the *bona fide* channel. We thus expressed MCU in two different heterologous systems, *Escherichia coli* and wheat germ cell-free transcription/translation,

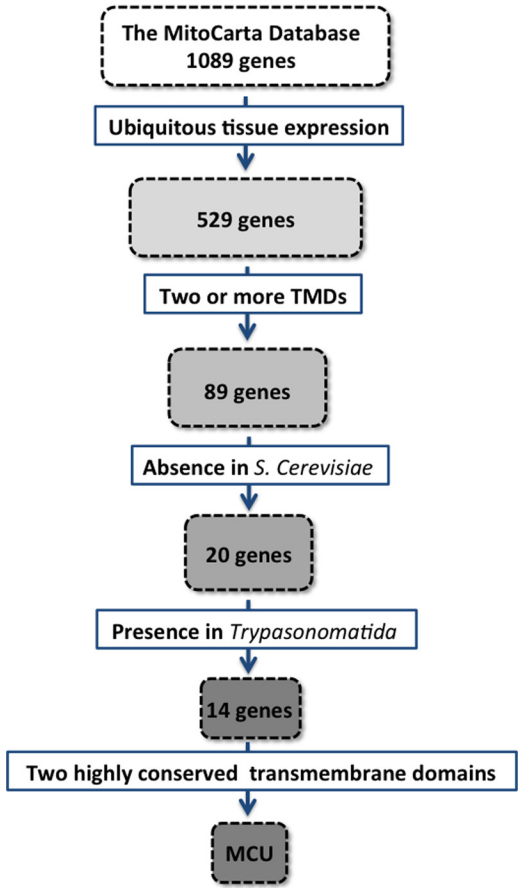

FIGURE 4. **Strategy for identifying MCU within the MitoCarta database.** *TMDs*, transmembrane domains.

and inserted the purified protein into the planar lipid bilayer. The electrophysiological properties of MCU matched those previously described in isolated mitochondria, and the current was completely inhibited by RuR and Gd^{3+}. MCU activity was also abolished by the mutation of two negatively charged residues to glutamine (D260Q/E263Q). Furthermore, when overexpressed in HeLa cells, this mutant reduced mitochondrial Ca^{2+} uptake, acting as a dominant negative, strongly suggesting that MCU forms an active channel as an oligomer (87). Mootha and co-workers (88) demonstrated that a single point mutation of MCU (S259A) retains function but confers resistance to Ru360, indicating that the most potent inhibitors of uniporter activity (RuR and related compounds) act directly on the channel and not with associated regulatory elements.

Conclusions

After a long search in the wrong directions, an unexpected twist rapidly unveiled the real identity of MCU. Now, the last element of the cellular calcium signaling toolkit has been identified, and the direct molecular targeting of mitochondrial Ca^{2+} homeostasis can be pursued *in vivo* and *in vitro*, as well as in organ physiology and disease pathogenesis. To cite only a few examples, the role of mitochondrial Ca^{2+} signals in aerobic metabolism and in tuning the autophagic response to nutrient deprivation can now be directly investigated and correlated with cell function and trophism, as in the case of muscle adaptation to feeding conditions, aging, or pathological states. In

contrast, sensitization to apoptotic and necrotic cell death can be evaluated in important disease models, such as ischemia-reperfusion of the heart and neurodegenerative disorders. Finally, future molecular data should include structural information supporting the discovery of specific inhibitors that may represent a novel important class of pharmacological compounds.

REFERENCES

1. Ringer, S. (1883) A third contribution regarding the influence of the inorganic constituents of the blood on the ventricular contraction. *J. Physiol.* **4,** 222–225
2. Grynkiewicz, G., Poenie, M., and Tsien, R. Y. (1985) A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* **260,** 3440–3450
3. Tsien, R. Y., Pozzan, T., and Rink, T. J. (1982) T-cell mitogens cause early changes in cytoplasmic free Ca^{2+} and membrane potential in lymphocytes. *Nature* **295,** 68–71
4. Miyawaki, A., Griesbeck, O., Heim, R., and Tsien, R. Y. (1999) Dynamic and quantitative Ca^{2+} measurements using improved cameleons. *Proc. Natl. Acad. Sci. U.S.A.* **96,** 2135–2140
5. Rudolf, R., Mongillo, M., Rizzuto, R., and Pozzan, T. (2003) Looking forward to seeing calcium. *Nat. Rev. Mol. Cell Biol.* **4,** 579–586
6. Cobbold, P. H., and Cuthbertson, K. S. (1990) Calcium oscillations: phenomena, mechanisms and significance. *Semin. Cell Biol.* **1,** 311–321
7. Hajnóczky, G., Robb-Gaspers, L. D., Seitz, M. B., and Thomas, A. P. (1995) Decoding of cytosolic calcium oscillations in the mitochondria. *Cell* **82,** 415–424
8. Dolmetsch, R. E., Xu, K., and Lewis, R. S. (1998) Calcium oscillations increase the efficiency and specificity of gene expression. *Nature* **392,** 933–936
9. Rizzuto, R., and Pozzan, T. (2006) Microdomains of intracellular Ca^{2+}: molecular determinants and functional consequences. *Physiol. Rev.* **86,** 369–408
10. Pozzan, T., Rizzuto, R., Volpe, P., and Meldolesi, J. (1994) Molecular and cellular physiology of intracellular calcium stores. *Physiol. Rev.* **74,** 595–636
11. Pinton, P., Pozzan, T., and Rizzuto, R. (1998) The Golgi apparatus is an inositol 1,4,5-trisphosphate-sensitive Ca^{2+} store, with functional properties distinct from those of the endoplasmic reticulum. *EMBO J.* **17,** 5298–5308
12. Calcraft, P. J., Ruas, M., Pan, Z., Cheng, X., Arredouani, A., Hao, X., Tang, J., Rietdorf, K., Teboul, L., Chuang, K. T., Lin, P., Xiao, R., Wang, C., Zhu, Y., Lin, Y., Wyatt, C. N., Parrington, J., Ma, J., Evans, A. M., Galione, A., and Zhu, M. X. (2009) NAADP mobilizes calcium from acidic organelles through two-pore channels. *Nature* **459,** 596–600
13. Berridge, M. J. (2001) The versatility and complexity of calcium signalling. *Novartis Found. Symp.* **239,** 52–64; discussion 64–77, 150–159
14. Vasington, F. D., and Murphy, J. V. (1962) Ca ion uptake by rat kidney mitochondria and its dependence on respiration and phosphorylation. *J. Biol. Chem.* **237,** 2670–2677
15. Deluca, H. F., and Engstrom, G. W. (1961) Calcium uptake by rat kidney mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* **47,** 1744–1750
16. Lehninger, A. L., Rossi, C. S., and Greenawalt, J. W. (1963) Respiration-dependent accumulation of inorganic phosphate and Ca ions by rat liver mitochondria. *Biochem. Biophys. Res. Commun.* **10,** 444–448
17. Mitchell, P., and Moyle, J. (1967) Chemiosmotic hypothesis of oxidative phosphorylation. *Nature* **213,** 137–139
18. Streb, H., Irvine, R. F., Berridge, M. J., and Schulz, I. (1983) Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate. *Nature* **306,** 67–69
19. Becker, G. L., Fiskum, G., and Lehninger, A. L. (1980) Regulation of free Ca^{2+} by liver mitochondria and endoplasmic reticulum. *J. Biol. Chem.* **255,** 9009–9012
20. Carafoli, E. (1967) *In vivo* effect of uncoupling agents on the incorporation of calcium and strontium into mitochondria and other subcellular fractions of rat liver. *J. Gen. Physiol.* **50,** 1849–1864
21. Rizzuto, R., Simpson, A. W., Brini, M., and Pozzan, T. (1992) Rapid changes of mitochondrial Ca^{2+} revealed by specifically targeted recombinant aequorin. *Nature* **358,** 325–327
22. Nagai, T., Sawano, A., Park, E. S., and Miyawaki, A. (2001) Circularly permuted green fluorescent proteins engineered to sense Ca^{2+}. *Proc. Natl. Acad. Sci. U.S.A.* **98,** 3197–3202
23. Rizzuto, R., Brini, M., Murgia, M., and Pozzan, T. (1993) Microdomains with high Ca^{2+} close to IP_3-sensitive channels that are sensed by neighboring mitochondria. *Science* **262,** 744–747
24. Szabadkai, G., Simoni, A. M., and Rizzuto, R. (2003) Mitochondrial Ca^{2+} uptake requires sustained Ca^{2+} release from the endoplasmic reticulum. *J. Biol. Chem.* **278,** 15153–15161
25. Montero, M., Alonso, M. T., Carnicero, E., Cuchillo-Ibáñez, I., Albillos, A., García, A. G., García-Sancho, J., and Alvarez, J. (2000) Chromaffin-cell stimulation triggers fast millimolar mitochondrial Ca^{2+} transients that modulate secretion. *Nat. Cell Biol.* **2,** 57–61
26. Rizzuto, R., Pinton, P., Carrington, W., Fay, F. S., Fogarty, K. E., Lifshitz, L. M., Tuft, R. A., and Pozzan, T. (1998) Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. *Science* **280,** 1763–1766
27. Csordás, G., Thomas, A. P., and Hajnóczky, G. (1999) Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria. *EMBO J.* **18,** 96–108
28. Szalai, G., Csordás, G., Hantash, B. M., Thomas, A. P., and Hajnóczky, G. (2000) Calcium signal transmission between ryanodine receptors and mitochondria. *J. Biol. Chem.* **275,** 15305–15313
29. Mannella, C. A., Buttle, K., Rath, B. K., and Marko, M. (1998) Electron microscopic tomography of rat-liver mitochondria and their interaction with the endoplasmic reticulum. *Biofactors* **8,** 225–228
30. Parekh, A. B., and Putney, J. W., Jr. (2005) Store-operated calcium channels. *Physiol. Rev.* **85,** 757–810
31. Glitsch, M. D., Bakowski, D., and Parekh, A. B. (2002) Store-operated Ca^{2+} entry depends on mitochondrial Ca^{2+} uptake. *EMBO J.* **21,** 6744–6754
32. David, G., Barrett, J. N., and Barrett, E. F. (1998) Evidence that mitochondria buffer physiological Ca^{2+} loads in lizard motor nerve terminals. *J. Physiol.* **509,** 59–65
33. Young, K. W., Bampton, E. T., Pinòn, L., Bano, D., and Nicotera, P. (2008) Mitochondrial Ca^{2+} signalling in hippocampal neurons. *Cell Calcium* **43,** 296–306
34. Giacomello, M., Drago, I., Bortolozzi, M., Scorzeto, M., Gianelle, A., Pizzo, P., and Pozzan, T. (2010) 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* **38,** 280–290
35. Csordás, G., Várnai, P., Golenár, T., Roy, S., Purkins, G., Schneider, T. G., Balla, T., and Hajnóczky, G. (2010) Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface. *Mol. Cell* **39,** 121–132
36. Hayashi, T., Rizzuto, R., Hajnóczky, G., and Su, T. P. (2009) MAM: more than just a housekeeper. *Trends Cell Biol.* **19,** 81–88
37. Szabadkai, G., Simoni, A. M., Chami, M., Wieckowski, M. R., Youle, R. J., and Rizzuto, R. (2004) Drp-1-dependent division of the mitochondrial network blocks intraorganellar Ca^{2+} waves and protects against Ca^{2+}-mediated apoptosis. *Mol. Cell* **16,** 59–68
38. de Brito, O. M., and Scorrano, L. (2008) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* **456,** 605–610
39. McCormack, J. G., Halestrap, A. P., and Denton, R. M. (1990) Role of calcium ions in regulation of mammalian intramitochondrial metabolism. *Physiol. Rev.* **70,** 391–425
40. Hansford, R. G. (1994) Physiological role of mitochondrial Ca^{2+} transport. *J. Bioenerg. Biomembr.* **26,** 495–508
41. Brini, M., Pinton, P., King, M. P., Davidson, M., Schon, E. A., and Rizzuto, R. (1999) A calcium signaling defect in the pathogenesis of a mitochondrial DNA inherited oxidative phosphorylation deficiency. *Nat. Med.* **5,** 951–954
42. Orrenius, S., Zhivotovsky, B., and Nicotera, P. (2003) Regulation of cell death: the calcium-apoptosis link. *Nat. Rev. Mol. Cell Biol.* **4,** 552–565
43. O'Rourke, B. (2000) Pathophysiological and protective roles of mitochondrial ion channels. *J. Physiol.* **529,** 23–36

44. Di Lisa, F., and Bernardi, P. (2009) A CaPful of mechanisms regulating the mitochondrial permeability transition. *J. Mol. Cell. Cardiol.* **46,** 775–780
45. Rasola, A., and Bernardi, P. (2011) Mitochondrial permeability transition in Ca^{2+}-dependent apoptosis and necrosis. *Cell Calcium* **50,** 222–233
46. Pinton, P., Ferrari, D., Magalhães, P., Schulze-Osthoff, K., Di Virgilio, F., Pozzan, T., and Rizzuto, R. (2000) Reduced loading of intracellular Ca^{2+} stores and downregulation of capacitative Ca^{2+} influx in Bcl-2-overexpressing cells. *J. Cell Biol.* **148,** 857–862
47. Pinton, P., Ferrari, D., Rapizzi, E., Di Virgilio, F., Pozzan, T., and Rizzuto, R. (2001) The Ca^{2+} concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the molecular mechanism of Bcl-2 action. *EMBO J.* **20,** 2690–2701
48. Palmer, A. E., Jin, C., Reed, J. C., and Tsien, R. Y. (2004) Bcl-2-mediated alterations in endoplasmic reticulum Ca^{2+} analyzed with an improved genetically encoded fluorescent sensor. *Proc. Natl. Acad. Sci. U.S.A.* **101,** 17404–17409
49. Foyouzi-Youssefi, R., Arnaudeau, S., Borner, C., Kelley, W. L., Tschopp, J., Lew, D. P., Demaurex, N., and Krause, K. H. (2000) Bcl-2 decreases the free Ca^{2+} concentration within the endoplasmic reticulum. *Proc. Natl. Acad. Sci. U.S.A.* **97,** 5723–5728
50. Scorrano, L., Oakes, S. A., Opferman, J. T., Cheng, E. H., Sorcinelli, M. D., Pozzan, T., and Korsmeyer, S. J. (2003) BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: a control point for apoptosis. *Science* **300,** 135–139
51. Murphy, A. N., Bredesen, D. E., Cortopassi, G., Wang, E., and Fiskum, G. (1996) Bcl-2 potentiates the maximal calcium uptake capacity of neural cell mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* **93,** 9893–9898
52. Cárdenas, C., Miller, R. A., Smith, I., Bui, T., Molgó, J., Müller, M., Vais, H., Cheung, K. H., Yang, J., Parker, I., Thompson, C. B., Birnbaum, M. J., Hallows, K. R., and Foskett, J. K. (2010) Essential regulation of cell bioenergetics by constitutive $InsP_3$ receptor Ca^{2+} transfer to mitochondria. *Cell* **142,** 270–283
53. Rizzuto, R., De Stefani, D., Raffaello, A., and Mammucari, C. (2012) Mitochondria as sensors and regulators of calcium signalling. *Nat. Rev. Mol. Cell Biol.* **13,** 566–578
54. Tinel, H., Cancela, J. M., Mogami, H., Gerasimenko, J. V., Gerasimenko, O. V., Tepikin, A. V., and Petersen, O. H. (1999) Active mitochondria surrounding the pancreatic acinar granule region prevent spreading of inositol trisphosphate-evoked local cytosolic Ca^{2+} signals. *EMBO J.* **18,** 4999–5008
55. Pivovarova, N. B., Hongpaisan, J., Andrews, S. B., and Friel, D. D. (1999) Depolarization-induced mitochondrial Ca accumulation in sympathetic neurons: spatial and temporal characteristics. *J. Neurosci.* **19,** 6372–6384
56. Chen, G., Racay, P., Bichet, S., Celio, M. R., Eggli, P., and Schwaller, B. (2006) Deficiency in parvalbumin, but not in calbindin D-28k, upregulates mitochondrial volume and decreases smooth endoplasmic reticulum surface selectively in a peripheral, subplasmalemmal region in the soma of Purkinje cells. *Neuroscience* **142,** 97–105
57. Jouaville, L. S., Ichas, F., Holmuhamedov, E. L., Camacho, P., and Lechleiter, J. D. (1995) Synchronization of calcium waves by mitochondrial substratesin *Xenopus laevis* oocytes. *Nature* **377,** 438–441
58. Hoth, M., Fanger, C. M., and Lewis, R. S. (1997) Mitochondrial regulation of store-operated calcium signaling in T lymphocytes. *J. Cell Biol.* **137,** 633–648
59. Rapizzi, E., Pinton, P., Szabadkai, G., Wieckowski, M. R., Vandecasteele, G., Baird, G., Tuft, R. A., Fogarty, K. E., and Rizzuto, R. (2002) Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca^{2+} microdomains to mitochondria. *J. Cell Biol.* **159,** 613–624
60. Madesh, M., and Hajnóczky, G. (2001) VDAC-dependent permeabilization of the outer mitochondrial membrane by superoxide induces rapid and massive cytochrome c release. *J. Cell Biol.* **155,** 1003–1015
61. Stock, D., Leslie, A. G., and Walker, J. E. (1999) Molecular architecture of the rotary motor in ATP synthase. *Science* **286,** 1700–1705
62. Cannon, B., and Nedergaard, J. (2004) Brown adipose tissue: function and physiological significance. *Physiol. Rev.* **84,** 277–359
63. Rizzuto, R., Bernardi, P., Favaron, M., and Azzone, G. F. (1987) Pathways for Ca^{2+} efflux in heart and liver mitochondria. *Biochemical J.* **246,** 271–277
64. Carafoli, E., Tiozzo, R., Lugli, G., Crovetti, F., and Kratzing, C. (1974) The release of calcium from heart mitochondria by sodium. *J. Mol. Cell. Cardiol.* **6,** 361–371
65. Palty, R., Silverman, W. F., Hershfinkel, M., Caporale, T., Sensi, S. L., Parnis, J., Nolte, C., Fishman, D., Shoshan-Barmatz, V., Herrmann, S., Khananshvili, D., and Sekler, I. (2010) NCLX is an essential component of mitochondrial Na^+/Ca^{2+} exchange. *Proc. Natl. Acad. Sci. U.S.A.* **107,** 436–441
66. Cox, D. A., Conforti, L., Sperelakis, N., and Matlib, M. A. (1993) Selectivity of inhibition of Na^+-Ca^{2+} exchange of heart mitochondria by benzothiazepine CGP-37157. *J. Cardiovasc. Pharmacol.* **21,** 595–599
67. Bernardi, P. (1999) Mitochondrial transport of cations: channels, exchangers, and permeability transition. *Physiol. Rev.* **79,** 1127–1155
68. Bernardi, P., Paradisi, V., Pozzan, T., and Azzone, G. F. (1984) Pathway for uncoupler-induced calcium efflux in rat liver mitochondria: inhibition by ruthenium red. *Biochemistry* **23,** 1645–1651
69. Mela, L. (1969) Inhibition and activation of calcium transport in mitochondria. Effect of lanthanides and local anesthetic drugs. *Biochemistry* **8,** 2481–2486
70. Bragadin, M., Pozzan, T., and Azzone, G. F. (1979) Activation energies and enthalpies during Ca^{2+} transport in rat liver mitochondria. *FEBS Lett.* **104,** 347–351
71. Kirichok, Y., Krapivinsky, G., and Clapham, D. E. (2004) The mitochondrial calcium uniporter is a highly selective ion channel. *Nature* **427,** 360–364
72. Michels, G., Khan, I. F., Endres-Becker, J., Rottlaender, D., Herzig, S., Ruhparwar, A., Wahlers, T., and Hoppe, U. C. (2009) Regulation of the human cardiac mitochondrial Ca^{2+} uptake by 2 different voltage-gated Ca^{2+} channels. *Circulation* **119,** 2435–2443
73. Panfili, E., Sandri, G., Sottocasa, G. L., Lunazzi, G., Liut, G., and Graziosi, G. (1976) Specific inhibition of mitochondrial Ca^{2+} transport by antibodies directed to the Ca^{2+}-binding glycoprotein. *Nature* **264,** 185–186
74. Ryu, S. Y., Beutner, G., Kinnally, K. W., Dirksen, R. T., and Sheu, S. S. (2011) Single channel characterization of the mitochondrial ryanodine receptor in heart mitoplasts. *J. Biol. Chem.* **286,** 21324–21329
75. Beutner, G., Sharma, V. K., Giovannucci, D. R., Yule, D. I., and Sheu, S. S. (2001) Identification of a ryanodine receptor in rat heart mitochondria. *J. Biol. Chem.* **276,** 21482–21488
76. Trenker, M., Malli, R., Fertschai, I., Levak-Frank, S., and Graier, W. F. (2007) Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca^{2+} uniport. *Nat. Cell Biol.* **9,** 445–452
77. Brookes, P. S., Parker, N., Buckingham, J. A., Vidal-Puig, A., Halestrap, A. P., Gunter, T. E., Nicholls, D. G., Bernardi, P., Lemasters, J. J., and Brand, M. D. (2008) UCPs–unlikely calcium porters. *Nat. Cell Biol.* **10,** 1235–1237; author reply 1237–1240
78. De Marchi, U., Castelbou, C., and Demaurex, N. (2011) Uncoupling protein 3 (UCP3) modulates the activity of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) by decreasing mitochondrial ATP production. *J. Biol. Chem.* **286,** 32533–32541
79. Jiang, D., Zhao, L., and Clapham, D. E. (2009) Genome-wide RNAi screen identifies Letm1 as a mitochondrial Ca^{2+}/H^+ antiporter. *Science* **326,** 144–147
80. Dimmer, K. S., Navoni, F., Casarin, A., Trevisson, E., Endele, S., Winterpacht, A., Salviati, L., and Scorrano, L. (2008) *LETM1*, deleted in Wolf-Hirschhorn syndrome, is required for normal mitochondrial morphology and cellular viability. *Hum. Mol. Genet.* **17,** 201–214
81. Dash, R. K., and Beard, D. A. (2008) Analysis of cardiac mitochondrial Na^+-Ca^{2+} exchanger kinetics with a biophysical model of mitochondrial Ca^{2+} handling suggests a 3:1 stoichiometry. *J. Physiol.* **586,** 3267–3285
82. Brand, M. D. (1985) The stoichiometry of the exchange catalysed by the mitochondrial calcium/sodium antiporter. *Biochem. J.* **229,** 161–166
83. Carafoli, E., Balcavage, W. X., Lehninger, A. L., and Mattoon, J. R. (1970) Ca^{2+} metabolism in yeast cells and mitochondria. *Biochim. Biophys. Acta* **205,** 18–26
84. Pagliarini, D. J., Calvo, S. E., Chang, B., Sheth, S. A., Vafai, S. B., Ong, S. E., Walford, G. A., Sugiana, C., Boneh, A., Chen, W. K., Hill, D. E., Vidal, M.,

Evans, J. G., Thorburn, D. R., Carr, S. A., and Mootha, V. K. (2008) A mitochondrial protein compendium elucidates complex I disease biology. *Cell* **134,** 112–123

85. Perocchi, F., Gohil, V. M., Girgis, H. S., Bao, X. R., McCombs, J. E., Palmer, A. E., and Mootha, V. K. (2010) *MICU1* encodes a mitochondrial EF hand protein required for Ca^{2+} uptake. *Nature* **467,** 291–296
86. Docampo, R., and Lukeš, J. (2012) Trypanosomes and the solution to a 50-year mitochondrial calcium mystery. *Trends Parasitol.* **28,** 31–37
87. De Stefani, D., Raffaello, A., Teardo, E., Szabò, I., and Rizzuto, R. (2011) A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. *Nature* **476,** 336–340
88. Baughman, J. M., Perocchi, F., Girgis, H. S., Plovanich, M., Belcher-Timme, C. A., Sancak, Y., Bao, X. R., Strittmatter, L., Goldberger, O., Bogorad, R. L., Koteliansky, V., and Mootha, V. K. (2011) Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. *Nature* **476,** 341–345