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INVITED REVIEW

Mitochondrial Ca^{2+} homeostasis: mechanism, role, and tissue specificities

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Abstract Mitochondria from every tissue are quite similar in their capability to accumulate Ca^{2+} in a process that depends on the electrical potential across the inner membrane; it is catalyzed by a gated channel (named mitochondrial Ca^{2+} uniporter), the molecular identity of which has only recently been unraveled. The release of accumulated Ca^{2+} in mitochondria from different tissues is, on the contrary, quite variable, both in terms of speed and mechanism: a Na^+-dependent efflux in excitable cells (catalyzed by NCLX) and a H^+/Ca^{2+} exchanger in other cells. The efficacy of mitochondrial Ca^{2+} uptake in living cells is strictly dependent on the topological arrangement of the organelles with respect to the source of Ca^{2+} flowing into the cytoplasm, i.e., plasma membrane or intracellular channels. In turn, the structural and functional relationships between mitochondria and other cellular membranes are dictated by the specific architecture of different cells. Mitochondria not only modulate the amplitude and the kinetics of local and bulk cytoplasmic Ca^{2+} changes but also depend on the Ca^{2+} signal for their own functionality, in particular for their capacity to produce ATP. In this review, we summarize the processes involved in mitochondrial Ca^{2+} handling and its integration in cell physiology, highlighting the main common characteristics as well as key differences, in different tissues.

Keywords Mitochondria · Ca^{2+} · MCU · NCLX

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The mitochondrial ability to accumulate Ca^{2+} in an energy-dependent process is firmly established since the early 1970s: both ATP (through reversal activity of the H^+ ATPase) or the respiratory chain (through H^+ pumping) can fuel mitochondrial Ca^{2+} uptake as they both generate a membrane potential, $\Delta\Psi$ (negative inside), across the inner mitochondrial membrane (IMM) that drives Ca^{2+} influx down its electrochemical gradient [99]. It was soon discovered that Ca^{2+} efflux mechanisms also exist (H^+/Ca^{2+} or Na^+/Ca^{2+} exchangers; for review see [76, 99]) that prevent reaching electrochemical equilibrium (incompatible with cell physiology). Indeed, without such efflux mechanisms, for a $\Delta\Psi$ of −180 mV, the Nernst equation would predict at equilibrium a mitochondrial $[Ca^{2+}]_{in}/[Ca^{2+}]_{out}$ ratio of about 10^6 fold, i.e., given a cytosolic Ca^{2+} concentration of 10^{-7} M at rest, the mitochondrial matrix $[Ca^{2+}]$ should be as high as 0.1 M. Accordingly, a general consensus was reached on a model in which the steady-state Ca^{2+} level within mitochondria of living cells is determined by a kinetic equilibrium between the influx of the cation [through the so-called mitochondrial Ca^{2+} uniporter (MCU)] and its efflux through the antiporters, with a Ca^{2+} futile cycle across the inner membrane of the organelles. The energy drain, due to the Ca^{2+} cycle, is however limited under resting conditions, thanks to the low affinity for Ca^{2+} of the MCU and, thus, the slow rates of Ca^{2+} influx and efflux under resting

conditions. It needs stressing, however, that any change in cytosolic Ca^{2+} concentration results in an alteration of this equilibrium and thus leads to an increase, or a decrease, of the steady-state mitochondrial matrix [Ca^{2+}].

Mitochondria isolated from every tissue tested, from liver to muscle, from brain to kidney, share these basic Ca^{2+} homeostatic features. While the kinetic characteristics of the Ca^{2+} uptake are quite similar in organelles isolated from various cells, the major difference among mitochondria from these tissues consists primarily in the mechanism and characteristics of the efflux pathways, mainly a H^+/Ca^{2+} exchanger in nonexcitable tissues (liver and kidney) and a Na^+/Ca^{2+} exchanger in excitable cells, e.g., neurons and striated muscles [37, 99]. Little attention, on the contrary, has been paid to the other key characteristic of every organelle involved in cellular Ca^{2+} homeostasis, i.e., the Ca^{2+} buffering capacity. One common Ca^{2+} buffering mechanism of mitochondria from all tissues depends on their ability to efficiently accumulate Pi (through a specific transporter); Ca^{2+}–Pi complexes form inside the mitochondrial matrix, favored by the alkaline pH of the matrix. Indeed, precipitates of Ca^{2+}–Pi have been revealed both upon massive Ca^{2+} accumulation into mitochondria of damaged cells and even under more physiological conditions [90]. Direct evidence for the formation of such complexes was obtained through the use of a Ca^{2+} surrogate, Mn^{2+}, that, once accumulated into the mitochondrial matrix through the MCU, forms Mn^{2+}–Pi complexes that can be easily monitored by their characteristic EPR spectrum [11]. On the contrary, no specific mitochondrial Ca^{2+}-binding proteins, equivalent to the endoplasmic (ER)/sarcoplasmic reticulum (SR) classical Ca^{2+} buffers (such as calsequestrin, calreticulin, Bip, etc.), have ever been described in mitochondria.

The Ca^{2+} toolkit of mitochondria

Although the functional characteristics summarized above have been discovered over 30 years ago, the Ca^{2+} toolkit responsible for Ca^{2+} uptake (the MCU) and release (the antiporters) has escaped molecular identification until very recently [37]. Several papers have been published in these three decades [61, 83, 84, 103, 106] claiming the identification of either of these proteins, but conclusive unambiguous identification has been obtained only in the last 2 years.

The mitochondrial Ca^{2+} uniporter

Although the Ca^{2+} uptake capacity of mitochondria was known since the late 1960s, the nature (a mobile carrier or an ion channel) of the MCU was unknown. In 1979, Bragadin et al., based on indirect data, proposed that the MCU is a gated ion channel [10], and this proposal was eventually directly confirmed in 2004 by Clapham and coworkers by patch clamping of the IMM [58]. A 40-kDa protein apparently capable of catalyzing Ca^{2+} uptake in reconstituted liposomes was isolated by Saris and coworkers in 1993 [103]. Whether or not this protein is related to the recently discovered MCU (whose MW is indeed ~40 kDa) is not known. In 2001, Beutner et al. showed that the classical ryanodine receptor, RyR, (type 1 isoform) can be isolated from the IMM of cardiac cells [9]. Immunostaining images with anti-RyR1-specific antibodies revealed the presence of gold particles in the mitochondrial membrane. RyR1 was found, however, only in mitochondria from heart (a tissue with strong expression in the SR membranes of bona fide type 2 RyR), while no RyR1 could be found in mitochondria isolated from other mammalian tissues (despite the fact that they take up Ca^{2+} from the medium as efficiently as cardiac mitochondria). The possibility that this finding was due to a contamination of the mitochondrial samples with SR membranes appears unlikely, as in heart cells RyR2, and not RyR1, is expressed. At the moment, however, this suggestion has not been followed by more convincing evidence.

The observation by Graier and coworkers [111] received lots of interest as it was suggested that the uncoupling proteins UCP2 and UCP3, believed to be involved only in H^+ permeability of the IMM, play a key role in Ca^{2+} uptake: their downregulation, in fact, reduced (while overexpression increased) the speed and amplitude of mitochondrial Ca^{2+} accumulation in living cells [111]. The authors suggested that UCP2 and 3, though not being themselves the MCU, are modulators of MCU activity. Of interest, the same group later reported that UCP2 and 3 affected the mitochondrial Ca^{2+} uptake due to ER Ca^{2+} release, but not due to capacitative Ca^{2+} influx [115]. The conclusions by Graier's group have been strongly challenged by other authors: among several arguments, the most relevant, in our opinion, is that purified liver mitochondria from $UCP2^{-/-}$ and $UCP3^{-/-}$ mice take up Ca^{2+} normally [13]. Demaurex and collaborators have demonstrated that UCP3 negatively modulates the activity of sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) by limiting mitochondrial ATP production [30]. Accordingly, they suggested that the effects of UCP3 on mitochondrial Ca^{2+} reflect metabolic alterations that impact on general cellular Ca^{2+} homeostasis. At the moment, this issue is on hold. Another possibility, not tested yet, is that UCP levels modulate the expression of the MCU itself.

In 2009, Clapham's group proposed that Ca^{2+} influx in mitochondria is catalyzed by two uptake systems, i.e., the classical MCU, endowed with low Ca^{2+} affinity, and a H^+/

Ca^{2+} electrogenic antiport with high Ca^{2+} affinity [56]. The latter was identified with the protein Letm1, previously believed to be a component of the H^+/K^+ exchanger of the plasma membrane [77] (for a recent review, see [78]). Noteworthy, purified Letm1 reconstituted in liposomes was able to catalyze a Ruthenium Red (RR)-sensitive Ca^{2+} uptake. Also this proposal, however, has been challenged by various groups on the basis of different experimental evidence. The most relevant, in our opinion, are: (1) yeasts, notoriously devoid of the capacity to accumulate Ca^{2+} within mitochondria in an energy-dependent way [18], express two orthologues of Letm1, Mdm38 and Mrs7 [104], and expression of human (or *Drosophila*) Letm1 rescues the yeast phenotype due to Mdm38 deletion [77]; (2) the yeast phenotype due to Mdm38-KO [and that of Letm1-KO mouse embryonic fibroblasts (MEF)] can be rescued by the addition of the ionophore nigericin, a bona fide H^+/K^+ exchanger [35, 79]. The role of Letm1 as an alternative high-affinity Ca^{2+} influx pathway in mammalian cells, thus, remains a subject of debate (see Nowikovsky K et al. for a recent review [78]), and more experimental evidence is required.

In 2010, Mootha and coworkers reported the identification of a protein, named MICU1, that appears to be a good candidate for MCU modulation [85]. Indeed, MICU1 is expressed ubiquitously in all eukaryotic cells tested thus far (from *Caenorhabditis elegans* to *Drosophila* to mammals), but not in yeasts; its downregulation results, at least under some conditions, in a substantial suppression of the Ca^{2+} uptake by mitochondria in intact (or in permeabilized) cells, though its overexpression had no overt effects. Most relevant, its predicted structure, one single membrane-spanning domain, suggests that MICU1 is rather a modulator of MCU than the uniporter itself.

Eventually, two groups in 2011 reported the identification of a protein, named this time MCU, that has all the characteristics predicted for the mitochondrial Ca^{2+} uniporter [4, 31]. The key findings in the two papers are: (1) the expression of MCU in different organisms is predicted, i.e., absent in yeast but expressed from *C. elegans* to plants and mammalian cells; (2) in mouse, MCU is expressed in every tissue tested; (3) reconstituted MCU in black lipid films leads to the appearance of ion channels that are permeable to Ca^{2+} with a single-channel conductance, similar to that found by Kirichok et al. [58] in patch clamped mitochondria, and inhibited by RR; (4) downregulation or overexpression of the protein leads to reduction or stimulation of mitochondrial Ca^{2+} uptake in intact and permeabilized cells; and (5) mutations in the predicted pore region result in a protein that behaves as dominant negative when recombinantly expressed in cells.

The study of MCU and MICU1 is still in its infancy, and many questions remain open. In particular, up to six isoforms of MCU are expressed in *Arabidopsis* with different tissue expression, but no evidence for a different function has been provided yet. In addition, MCU and MICU1 have been reported to form complexes in the IMM, but the functional role of this interaction is not clear.

Regarding the structure of the functional mitochondrial uniporter, the topology of the single polypeptide is still debated and, although circumstantial evidence has been provided suggesting that the MCU pore forming structure is a multimer of the protein, this proposed model is waiting for more definitive direct evidence. It is easy to predict that these basic questions will soon be answered, perhaps even before this contribution is published.

The antiporters

Similarly, the identification of the molecular identity of the Na^+/Ca^{2+} and H^+/Ca^{2+} exchangers has been complex. The proposal that mitochondria are endowed with Ca^{2+} efflux pathway(s) goes back to the 1970s when it was discovered that, in isolated mitochondria, addition of RR, after Ca^{2+} had been accumulated, results in a slow and practically complete release of the cation into the medium [26, 92, 93]. It was suggested that, in liver mitochondria, such efflux is due to a 2 H^+/Ca^{2+} electroneutral process [26, 93], while in mitochondria from heart and other excitable cells, such efflux is catalyzed by a Na^+-dependent process and, accordingly, the existence of a Na^+/Ca^{2+} exchanger, similar to that known to exists in the plasma membrane, was postulated [26]. Initially, the proposal was that, the mitochondrial Na^+/Ca^{2+} exchange was electroneutral, but the consensus was soon reached that it is, as in the case of the plasma membrane homologue, electrogenic with three or four Na^+ ions exchanged per one Ca^{2+}[57]. The net gain in matrix Na^+ is then compensated by the Na^+/H^+ exchanger, another antiporter known to be expressed by mitochondria, whose molecular identity, however, is still unknown. Again, several attempts have been made to isolate the two antiporters: Li et al. [61] and Paucek and Jaburek [84] reported the isolation of a partially purified protein fraction from the IMM of the heart that catalyzed a Na^+-dependent Ca^{2+} transport in reconstituted liposomes. However, the gene responsible for such transport was not identified. In 2010, Sekler and coworkers reported that one isoform of the classical plasma membrane Na^+/Ca^{2+} exchanger family, the NCLX, until then poorly characterized, is the only member endowed with a functional characteristic of the Na^+/Ca^{2+} exchanger of mitochondria: the capacity of exchanging Ca^{2+} not only with Na^+ but also with Li^+. Moreover, NCLX was shown to be highly enriched in the

mitochondrial fraction, while it was absent from plasma membrane-enriched microsomes [82]. Downregulation of NCLX practically abolished the capacity of isolated mitochondria to extrude Ca^{2+} in a Na^+-dependent pathway, while overexpression of NCLX had the opposite effect. In addition, selective mutations in its membrane-spanning domain result in the generation of a dominant-negative construct, suggesting that the functional exchanger results from oligomerization of multiple subunits. Although our biased opinion is that NCLX does indeed represent the long searched for Na^+/Ca^{2+} exchanger of the IMM, a number of questions remain open. In particular, is the differential activity of Na^+/Ca^{2+} exchange found in different cells solely due to different NCLX expression levels or is it also due to posttranslational modifications? This question arises from the observation that the NCLX mRNA appears to be expressed at relatively homogeneous levels in different tissues, while the translated protein (as revealed by Western blotting), at least in rat, is particularly abundant in the exocrine pancreas, skeletal muscle, stomach and, to lesser extent, in cardiac tissue, brain, spleen, and skin. Most relevant, the NCLX tissue level (in whole homogenates) does not correlate with the activity of the Na^+/Ca^{2+} exchange of mitochondria isolated from different tissues. Also, is NCLX only localized in the IMM? In the original report by Cai and Lytton [15], NCLX was enriched in the microsomal and plasma membrane fractions, while according to Palty et al., the endogenous NCLX is recovered almost exclusively in the mitochondrial fraction [82]. It is worth mentioning also that two alternatively spliced isoforms of NCLX were found in humans, a long and a short isoform [81]. Once reconstituted, the long and the short isoform catalyzed equivalent activity of Na^+/Ca^{2+} exchange. Noteworthy, the intensity of the two bands revealed by the antibody in Western blotting (at about 75 and 55 KDa) varies substantially depending on the tissue.

In a number of cells, most notably in hepatocytes, the activity of the Na^+-dependent Ca^{2+} efflux is extremely low, while most of the efflux depends on a H^+/Ca^{2+} exchange. As to this latter, although a partially purified protein of 66 kDa endowed with exchange activity when reconstituted in liposomes has been described [113], the situation is still quite confusing. Not only the encoding gene has not been unraveled yet but several of its functional properties remain undetermined. For example, it is presently still unclear whether the exchanger is intrinsically electroneutral or electrogenic (2 H^+ or 3 H^+/Ca^{2+}), though evidence that it can be modulated by membrane potential has been provided [8].

Last, but not least, Ca^{2+} efflux may take place through the so-called permeability transition pore (PTP). Owing to its large size, the PTP could provide the mitochondria with a fast Ca^{2+} release channel, preventing matrix Ca^{2+} overload, an idea supported by some studies (for review see [7]) and by the recent identification in *Drosophila melanogaster* of a H^+-permeant Ca^{2+}-release channel with features intermediate between those of the PTP of yeast and mammals [114]. However, the PTP opening also induces the collapse of membrane potential and, accordingly, Ca^{2+}, in depolarized organelles, could be released also through the MCU.

Characteristics of Ca^{2+} handling by mitochondria in different cells

The characteristics briefly summarized above apply to purified mitochondria from all types of mammalian cells (and also to mitochondria from plants). However, substantial differences have been found in organelles purified from different tissues, the most important being: (1) in heart mitochondria, pharmacological evidence has been provided indicating the existence of two Ca^{2+} import mechanisms, both sensitive to RR, but with different affinities for this drug [71]; (2) RyR-like channels have been shown to exist only in mitochondria of the heart muscle [9]; and (3) though all mitochondria possess a Ca^{2+} efflux mechanism, the Na^+-dependent one tends to be most active, though not exclusive, in excitable cells [26]. Given that the molecular identities of the MCU, MICU1, NCLX have been unraveled only very recently, the information on tissue expression of these molecules is still fragmentary. To the best of our knowledge, all these proteins are ubiquitously expressed, though significant differences in the levels of the mRNAs (at least for MCU) have been reported [31]. These differences may simply reflect either the abundance of mitochondria in the tissue, the turnover of the organelle protein, or both.

As to other characteristics relevant for mitochondrial Ca^{2+} handling in different tissues, they concern primarily in the relative abundance of the organelles; in their subcellular distribution and, in particular, their relationships with ER/SR and with the plasma membrane; and in their shape, i.e. elongated vs rounded organelles. A complete overview of these three aspects is far beyond the scope of this work, and the reader is invited to consult the vast body of literature available, including a few recent reviews [17, 36, 37, 91, 105]. Here, we will limit ourselves to a few examples concerning mammalian cells.

In cell types that primarily depend on oxidative metabolism for ATP synthesis, e.g., neurons, red skeletal muscle fibers, or cardiac myocytes, mitochondria can account for up to 30 % of the cytoplasmic volume. It is not surprising, therefore, that molecular defects in mitochondria primarily affect the brain and muscles. Other organs, such as the liver and kidney, also possess a large percentage of mitochondrial proteins per total amount of protein and rely heavily on mitochondria for supporting ATP-dependent functions. Despite this, genetic defects of mitochondria do not result in major functional deficits in these organs. Noteworthy, also cells that are not often considered particularly enriched in mitochondria, such as T lymphocytes, may primarily depend on oxidative phosphorylation for their ATP production. Indeed, recent evidence suggests that mitochondria may serve a key function in the activation of lymphocytes by antigens or chemokines [16, 23].

The question of whether there is a positive correlation between the mitochondrial mass and their importance in Ca^{2+} homeostasis remains, on the contrary, unsettled. For example, in cardiac myocytes, it is still debated whether or not mitochondria take up and release Ca^{2+} on a "beat-to-beat" basis and, most relevant, whether they significantly contribute to buffering systolic Ca^{2+} changes (see below).

As to the mitochondrial distribution within the cell and their relationships with other organelles, the situation is quite different in different cells. We briefly discuss the relationship between SR/ER and then the apposition of mitochondria to the plasma membrane.

In striated muscle, the subcellular distribution of all intracellular organelles is largely, but not exclusively, dictated by the organization of the myofibers and of the plasma membrane invaginations, the T tubules. Franzini-Armstrong argued that, in skeletal muscle, although mitochondria are closely tethered to the SR terminal cisternae (Fig. 1a), the RyRs are localized at some distance from mitochondria (about 100 nm) as these Ca^{2+} release channels are located on the SR surface facing the T tubules [40]. A similar situation applies also to cardiac cells (Fig. 1b), though the SR cisternae are flatter, and thus, the nearest distance between RyRs and the outer mitochondrial membrane (OMM) is shorter (around 30 nm). She thus correctly concluded that "Because the SR lumen separates RyRs from the mitochondrial outer surface, there is no chance for a direct molecular link between the RyRs and the mitochondrion. It must therefore be assumed that mitochondrial Ca^{2+} uptake in muscle depends on diffusion of Ca^{2+} released from the SR to the mitochondrial surface" [40].

A unique case in terms of subcellular localization is that of pancreatic acinar cells, where mitochondria are distributed as a belt below the granule area. This localization appears essential to form a barrier to the diffusion of Ca^{2+}, released by the ER cisternae present in the apical region, towards the nuclear and basolateral part of the cell [86].

As to other cell types, there is no obvious pattern of mitochondrial sublocalization: the organelles have an apparent random distribution, being present around the nuclear membrane, in the bulk cytosol, and often close to the plasma membrane. The most studied aspect of the subcellular localization of the mitochondria is their relationship with the ER and in particular with the IP_3 receptors. Close appositions of mitochondria to ER cisternae are indeed found not only in cardiac or skeletal muscles but also in most cell types investigated so far (Fig. 2a). Tethering structures connecting mitochondria to ER have been described in practically every cell type (for a recent review, see [48]). Recent evidence suggests

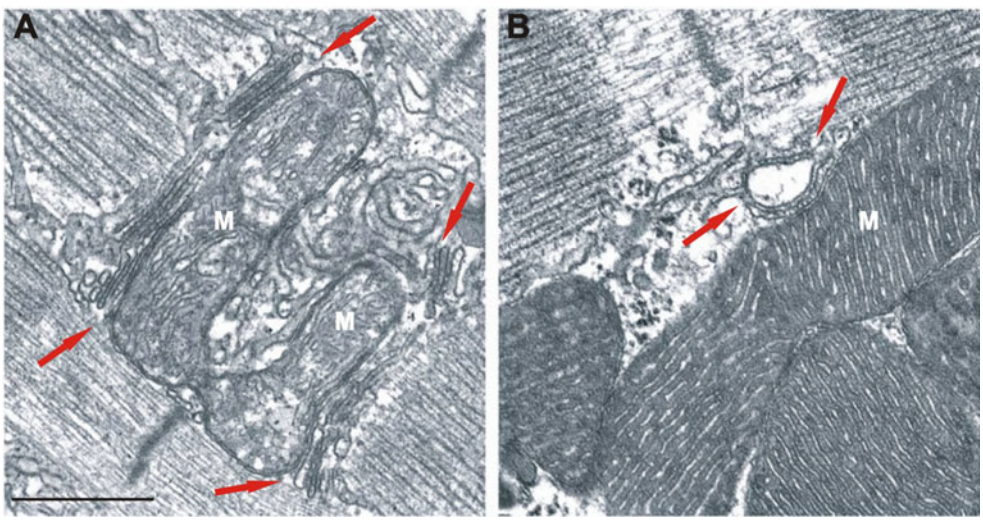

Fig. 1 Close apposition between SR Ca^{2+} release sites and mitochondria in adult mammalian striated muscles. Electron micrograph of mouse fast skeletal muscle fibers (mitochondria rich) (**a**) and cardiac muscle cells (**b**). Mitochondria (*M*) appear in close proximity to Ca^{2+} release units (triads or dyads, *arrows*). *Bar*, 0.5 μm. The figure is modified, with permission, from Ref. [45]. © 2007 American Physiological Society

that mitofusin 2 is a key player in the formation of such ER/mitochondrial close appositions. Mitofusin 2 is localized both in the outer mitochondrial membrane and on the ER surface [29]. Downregulation or overexpression of mitofusin 2, in fact, results in reduction or increase in the formation of ER/mitochondrial junctions, respectively [29]. Although the genetic manipulation of mitofusin 2 cellular levels has, by itself, some effects on ER Ca^{2+} handling, evidence supports the idea that mitofusin 2 KO results in a reduction of the ER to mitochondria Ca^{2+} transfer. The opposite also appears to be true [29].

We have recently reported that a protein expressed in the ER membrane (and also in the Golgi and plasma membrane), presenilin 2 (PS2), but not its close homologue presenilin 1 (PS1), favors the apposition of mitochondria to ER, as well as their Ca^{2+} cross-talk (Fig. 2b) [118]. This phenomenon requires mitofusin 2 expression (Filadi et al., unpublished result), but does not depend on an effect of PS2 on mitofusin 2 protein levels. Of note, both PS1 and PS2 are found enriched in the so-called mitochondria-associated membranes (MAMs), domains of close apposition between ER and mitochondria [3]. Interestingly, in PS2 KO MEF cells (but not in PS1 KO cells), mitochondrial dysfunctions have been reported, such as reduced mitochondrial membrane potential and lower respiration rate [5]. This energy deficit could indeed depend on the reduction of ER–mitochondria Ca^{2+} transfer, due to a decreased organelle apposition caused by the absence of PS2. As recently demonstrated in other cell models, in fact, the constitutive Ca^{2+} transfer occurring in resting conditions between the two organelles is critical to maintain mitochondrial functions, to avoid mitophagy and to ensure proper ATP production ([19] and see below). In addition, and most relevant from a pathogenic point of view, the increased mitochondria–ER tethering caused by PS2 overexpression is more marked in cells expressing the mutant forms of PS2 (Fig. 2b), i.e., those responsible for familial Alzheimer's disease [118, 119].

The interest for mitochondria–ER interactions is due to the fact that these regions are believed to be particularly relevant for two key physiological phenomena, i.e., the exchange of lipids between the ER and mitochondria and the Ca^{2+} uptake by mitochondria upon release from ER channels. This latter aspect is an extensively reviewed field, and we refer the reader to some recent reviews on the topic for a more thorough discussion of this point [24, 40, 44, 91, 99]. Here, we wish to point out only a general comment. The fast uptake of Ca^{2+} by mitochondria, upon release from the ER/SR, is dependent on these close appositions since only when mitochondria are close to the release Ca^{2+} channels the local $[Ca^{2+}]$ does reach sufficiently high values to overcome the intrinsic low affinity of the MCU for Ca^{2+}; these Ca^{2+} hot spots cover a significant part of the OMM surface, and the $[Ca^{2+}]$ reached in these regions can be as high as 20–30 μM (Fig. 2b). However, the generation of local Ca^{2+} hot spots on the surface of mitochondria is necessary for triggering *fast* mitochondrial Ca^{2+} uptake, but it is not necessary for allowing *any* Ca^{2+} uptake by the organelles. Indeed, slow Ca^{2+} uptake into the organelles will occur under any circumstance that induces an elevation in cytosolic Ca^{2+} (see above), and it is in fact constantly occurring also under resting conditions. In this latter case, the slow uptake rate is balanced by the release through the antiporters, and no net mitochondrial Ca^{2+} accumulation occurs, but there is only a futile cycling of Ca^{2+} across the inner membrane. Recently, it has been suggested that also in "resting" cells a continuous fast uptake of Ca^{2+} occurs in a few mitochondria upon spontaneous or induced (by low level of stimulation) opening of IP_3 receptors ([19] and see below).

Mitochondria are often also closely associated to the plasma membrane. The mechanisms that bring mitochondria to this location are presently unknown. Tethering structures, similar to those described to link mitochondria to ER/SR, have not been described for the plasma membrane, but characteristic locations of mitochondria below the plasmalemma are particularly evident in a few cell types such as smooth muscle [42] and pancreatic acinar cells [110]. While the localization of mitochondria close to the ER/SR appears consistent with the necessity of exchanging lipids between the two organelles, to fuel ATP to the sarco–endoplasmic reticulum Ca^{2+} ATPases, SERCAs, and/or to take Ca^{2+} released from the ER/SR, the need for mitochondria close to the plasma membrane has been less investigated. The most obvious role of mitochondrial apposition to the plasma membrane appears to be the modulation of the activity of Ca^{2+} channels.

In neurons, mitochondria are found in the cell body, in the processes (both along the axon and dendrites; Fig. 2a), and also in the pre- and postsynaptic regions. As to the latter subcellular compartments, the distance of mitochondria from the plasma membrane is usually relatively large (about 0.5 μm) and, accordingly, it is unlikely that they are exposed to the high Ca^{2+} microdomains generated in the "active zones" upon opening of presynaptic voltage-gated Ca^{2+} channels (VOCCs) or close to the channel mouths upon activation of postsynaptic Ca^{2+}-permeable channels (VOCCs, NMDA receptors, AMPA receptors, etc.). Thus, in these locations, it appears unlikely that mitochondria can modulate either the Ca^{2+}-dependent inactivation of pre- or postsynaptic Ca^{2+} channels, or the activation of vesicle

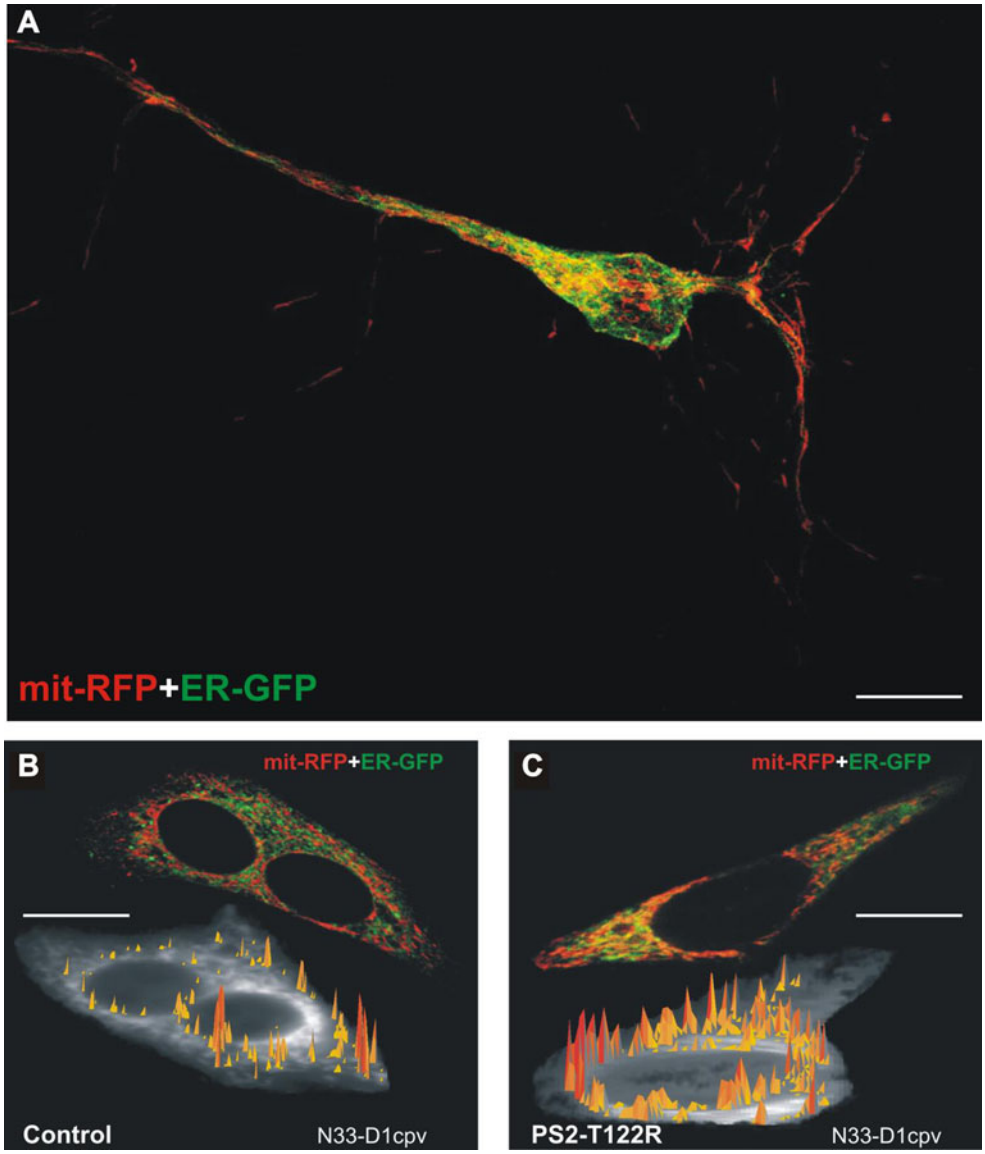

Fig. 2 Structural and functional ER–mitochondria interactions in neuronal cells. **a** Confocal merged image of a mouse cortical neuron transiently co-transfected with a mitochondrial RFP (mit-RFP) (*red*) and an ER-targeted GFP (ER-GFP) (*green*). In this image, the regions of ER–mitochondria apposition are stained in *yellow*. **b**, **c** Confocal merged images (*upper cell in each panel*) of neuroblastoma SH-SY5Y cells transiently co-transfected with cDNAs coding for a familial Alzheimer's disease (FAD)-PS2 mutant (PS2-T122R; C) or void vector (control, **b**), together with those coding for ER-GFP (*green*) and mit-RFP (*red*). As in **a**, the regions of ER–mitochondria apposition are stained in *yellow*. PS2-T122R-expressing cells show increased ER–mitochondria interactions. In the *lower cells* of **b** and **c**, SH-SY5Y cells were co-transfected with cDNAs coding for the FAD-PS2 mutant (**b**) or void vector (control, **a**) and the Ca^{2+} probe N33-D1cpv, to measure the Ca^{2+} concentration on the outer mitochondrial membrane (OMM). Live cells were challenged with the IP_3-generating stimulus bradykinin, and the Ca^{2+} microdomains generated on the surface of OMM are presented in pseudocolors as 3D spikes. The amplitude of the Ca^{2+} spikes is coded (as indicated in the *bar on the right*) from *yellow to red*, low and high Ca^{2+}. For a similar average cytosolic Ca^{2+} rise, the Ca^{2+} microdomains on the OMM are higher and more abundant in FAD-PS2-expressing cell compared to controls. See reference [49] for details. *Bar*, 10 μm. Figure 2A is modified, with permission, from "Endoplasmic Reticulum-mitochondria connections, calcium cross-talk and cell fate: a closer inspection." P. Agostinis, A. Samali (eds), "Endoplasmic Reticulum Stress in Health and Disease", DOI 10.1007/978-94-007-4351-9_4, @ 2012 Springer Science+Business Media Dordrecht. Figure 2B is modified, with permission, from Ref. [53]. © 2011 Landes Bioscience

release. However, Ca^{2+} channels in neurons are located also in other domains of the plasma membrane, and no obvious barrier to the proximity of mitochondria to the plasma membrane appears to exist in these regions. Accordingly, mitochondria, if sufficiently close to the plasma membrane, can be exposed to local hot spots of high [Ca^{2+}] at the channel mouths. Consequently, mitochondria can modulate the channel properties and undergo a massive Ca^{2+} accumulation, the so-called Ca^{2+} overload. This may be particularly relevant, for example, during activation of extrasynaptic

NMDA channels, often triggering a mitochondrial-dependent Ca^{2+} toxicity. In some neuroendocrine cells, e.g., adrenal medulla cells, a major mitochondrial Ca^{2+} accumulation has been measured upon opening of Ca^{2+} VOCCs [74]. In this cell type, a mitochondrial depolarization drives, in a subfraction of mitochondria, massive increases in matrix [Ca^{2+}] (up to 5–600 μM) [74]. Similar results have been obtained in GH3 cells [43].

Regarding the relationship between mitochondrial Ca^{2+} handling and organelle's shape, there is no evidence that elongated vs round mitochondria have distinct Ca^{2+} uptake properties. However, fragmentation of the mitochondrial network may substantially modify the organelle Ca^{2+} uptake, for example by changing their interaction with other intracellular membranes, such as the ER and plasma membrane. For example, fragmentation of mitochondria blocked the propagation of the Ca^{2+} spread within the network, increasing the heterogeneity of Ca^{2+} elevations among organelles in the same cell [109]. Moreover, Fis1-mediated mitochondrial fission in HeLa cells caused not only fragmentation of the network but also mitochondria redistribution from the cell periphery towards the perinuclear space [41], favoring their more extensive connection with the ER Ca^{2+}source [108]. Often, the fragmentation of mitochondria and the collapse of their $\Delta\Psi$ accompany or anticipate cell damage; accordingly, it is not surprising that under these conditions, small round organelles do not efficiently uptake Ca^{2+}. The reduced Ca^{2+} uptake, however, is due to the $\Delta\Psi$ drop and not to the shape change. The functional role of mitochondrial shape has been extensively reviewed recently (see for example [36, 105]).

Role of Ca^{2+} uptake by mitochondria

While the capacity of mitochondria to accumulate Ca^{2+} into their matrix under physiological conditions is now undisputed, far less clear is its functional role. Three major functions have been considered: (1) buffering local or bulk cytosolic Ca^{2+} rises; (2) activation of matrix dehydrogenases and, accordingly, electron flow through the respiratory chain and ATP synthesis; and (3) activation of cell death. The latter aspect will not be dealt with here since a number of recent reviews exist for the interested reader (see, for example, [27, 32, 38, 44, 80, 89, 96, 98]).

Analyzing the studies carried out to causally link mitochondrial Ca^{2+} uptake to specific cell functions, the major problem that emerges is that they are all based on positive (or negative) correlations or on the use of drugs whose specificity is often dubious. Inhibition of mitochondrial Ca^{2+} uptake has been obtained primarily in two ways: using uncouplers (or a combination of inhibitors of the respiratory chain and of the H^+ ATPase) that collapse mitochondrial membrane potential or blocking the Ca^{2+} uniporter with RR or Ru360. Both these approaches are subject to major criticisms: in the case of drugs that collapse mitochondrial membrane potential, several other aspects of cellular metabolism are, or may be, altered, i.e., cellular ATP levels, cytosolic pH, and plasma membrane potential. As to RR or Ru360, in most cases they are applied extracellularly, with the assumption that they penetrate into live cells. Although prolonged incubations of intact cells with RR or Ru360 lead to net cellular accumulation of the drugs [65], whether these drugs are free in the cytoplasm or solely entrapped within endosomes is debated and may depend on the cell type. A careful study [49] has shown that in various cell types in culture, the observed effects of these drugs are presumably due to modifications of plasma membrane proteins rather than to inhibition of the MCU. In our experience, by directly measuring Ca^{2+} uptake in mitochondria of intact living cells, we never observed an inhibition of mitochondrial uptake by RR or Ru360 [100]. In a very few cases, RR was injected intracellularly [63]; however, also in these cases, the possibility that RR affects functions other than mitochondrial Ca^{2+} uptake (e.g., RyR functionality) cannot be completely ruled out. With these caveats in mind, different results have been obtained in different model systems, depending on the parameter investigated, and the question on what is the physiological role for mitochondrial Ca^{2+} uptake remains largely unsolved.

Local Ca^{2+} buffering

As to local Ca^{2+} buffering, we here refer to the capacity of mitochondria localized in close proximity to the channel mouth to uptake part of the Ca^{2+} flowing through the channels themselves. The effect necessarily requires a very short distance of mitochondria from the Ca^{2+} channels (10–30 nm) and sufficient speed of the mitochondrial MCU to cope with the diffusion of Ca^{2+} away from the channel mouth. As to the first, evidence for ER/SR mitochondrial apposition has been provided by several groups (see above), and the formation of hot spots on the OMM (Fig. 2b) has been experimentally demonstrated [43, 28]. Several data reported that MAMs are particularly enriched with the type 3 of the IP_3 receptor [52, 70] and that the chaperone Sigma-1 receptor plays a role in the generation of this channel clustering [52]. Given that local Ca^{2+} at the mouth of the IP_3 receptors results in either facilitation or inhibition of channel opening (depending on the isoform involved [72]), the speed and amplitude of IP_3-induced Ca^{2+} release have been investigated under control conditions and when mitochondria were prevented (using uncouplers) from taking up Ca^{2+}. Evidence has thus been obtained indicating that indeed mitochondrial Ca^{2+} uptake can positively or negatively modulate the efficacy of IP_3 receptor-dependent Ca^{2+} release [50, 59]. In addition, in some cellular models, the modulation

of the local [Ca^{2+}] by mitochondria apposed to IP_3 receptors appears to be essential for controlling the development, maintenance, and frequency of cytosolic Ca^{2+} oscillations [120].

A question still open to debate is whether or not the OMM represents a kinetic barrier to the diffusion of Ca^{2+} into the intermembrane space and, thus, to the MCU facing this region. The permeability of the OMM to Ca^{2+} is high, and a Ca^{2+} probe (aequorin) localized in the intermembrane space failed to reveal significant differences in the [Ca^{2+}] between this region and the bulk cytosol when permeabilized cells were exposed to different Ca^{2+} levels [97]. The Ca^{2+} permeability of the OMM is most likely insured by the so-called voltage-dependent anion channel (VDAC); Rizzuto and coworkers have shown that overexpression of VDAC results in a significant increase in the speed and amplitude of IP_3-dependent Ca^{2+} uptake by mitochondria [95]. In turn, this result suggests that, at least in the regions of close apposition between the ER and mitochondria, Ca^{2+} permeation through VDAC may represent the rate-limiting step for MCU-dependent Ca^{2+} uptake into the matrix.

The situation is somehow more confusing as far as plasma membrane Ca^{2+} channels are concerned. It has been proposed that mitochondria can modulate both the Ca^{2+} inactivation of NMDA receptors [14] and that of capacitative Ca^{2+} influx (due to Orai1 channels; [53, 94]. However, we have recently demonstrated that, while fast massive Ca^{2+} accumulation can be monitored upon opening of VOCCs in a subpopulation of mitochondria apposed to the plasma membrane, no such fast accumulation can be observed when Ca^{2+} enters through Orai1 channels [43]. Indeed, given that the latter Ca^{2+} influx pathway requires the physical apposition of the plasma membrane to ER cisternae (and the physical interaction of Orai1 channels with the ER protein STIM1 [20, 62]), it is not surprising that mitochondria cannot get very close to the open channels and must be located at a few hundred nanometers away from them. Accordingly, though the organelles can experience a local [Ca^{2+}] higher than that of the bulk cytosol, we concluded that they cannot get close enough to buffer the Ca^{2+} concentration at the very mouth of the channels themselves. In partial contrast with this conclusion, Hoth and coworkers [94] provided evidence supporting an important role of mitochondrial Ca^{2+} uptake in modulating Orai1-dependent Ca^{2+} influx in T lymphocytes. Again, a general limitation of these studies is that they are largely based on indirect or pharmacological approaches. The discovery of the MCU and the possibility of modifying genetically its expression levels will now offer more direct tools to address these questions.

Buffering bulk cytoplasmic Ca^{2+}

As to buffering Ca^{2+} changes in the bulk cytoplasm, although it is an apparently more straightforward problem, the results are profoundly different in the various cell types and often have been contradictory. A first consideration to take into account is the mitochondrial volume. As pointed out above, this parameter may be highly variable: it may represent 2–5 % of the cytoplasmic volume (and be equivalent to that of the ER) in several cultured cells or non-muscle cells, while in red skeletal muscle fibers and in cardiac muscle, the mitochondrial volume can be, respectively, 5- and 16-fold higher than that of the SR [39]. Accordingly, at least in theory, in these cells the uptake of even a small fraction of released Ca^{2+} by mitochondria may contribute significantly to buffering cytoplasmic Ca^{2+} increases. Noteworthy, in red (mitochondria rich) skeletal muscle fibers, most of the mitochondria are also in close proximity to Ca^{2+} release sites (for review see [40]). It has been also demonstrated that RR application to skinned red fibers (but not to mitochondria poor fibers) affects their relaxation rate [45].

Much debated and, at present, still unsolved is the role of mitochondrial Ca^{2+} uptake in buffering the peak Ca^{2+} rises in cardiac cells. For some time, it was debated whether or not mitochondria were sufficiently fast to take up part of the Ca^{2+} released from the SR during systole. While the consensus has increased in support of a "beat-to-beat" Ca^{2+} oscillation within mitochondria, it remains unknown what percentage of the total Ca^{2+} is sequestered by mitochondria in cardiomyocytes. However, based on indirect data, Subramanian et al. [107] showed that, in cardiac muscle from rats, mitochondria can affect the transverse propagation of Ca^{2+} waves by buffering Ca^{2+} at the level of the Z line. The possibility, now opened, to either downregulating (via siRNA) or overexpressing MCU will allow to address these question in a genetic, less ambiguous way. Unpublished observation by us (Drago et al.) demonstrates indeed that in neonatal cardiac myocytes, genetic MCU modulation has drastic effects on the amplitude of Ca^{2+} peaks during spontaneous Ca^{2+} oscillations, with a downregulation of MCU increasing, and its overexpression decreasing, the amplitude of the systolic Ca^{2+} peaks.

In sympathetic neurons [22] or in adrenal medulla cells [74], on the contrary, massive Ca^{2+} increases (as induced by K^+-dependent depolarization or by long trains of action potentials) are substantially buffered by mitochondria: indeed, addition of uncouplers results in large increases in the peak cytosolic Ca^{2+} and (in the adrenal medulla cells) massive stimulation of secretion [73]. In pancreatic acinar cells, blockade of mitochondrial Ca^{2+} uptake allows the diffusion of locally restricted apical Ca^{2+} increases into the basolateral region of the cells [110]. It has been suggested that in these cells, impairment of the Ca^{2+} buffering capacity by mitochondria may be causally important under pathological conditions, e.g., in alcoholism. In this case, excessive alcohol intake may inhibit pancreatic mitochondrial function,

followed by prolonged Ca^{2+} signals diffuse to the whole cell, trypsin activation within the secretory granules, and pancreatitis [87, 88].

In many other cell types, e.g., pancreatic β cells and various cultured cell lines, the collapse of mitochondrial membrane potential usually has small effects on the amplitude of cytoplasmic Ca^{2+} rises elicited by ER Ca^{2+} mobilization (see for example [97]). These data, as discussed above, need to be considered cautiously due to the side effects of the drugs employed. Up until now, the only published study (carried out with genetic manipulation of MCU levels) addressing the role of mitochondria in bulk cytosolic Ca^{2+} buffering is that of De Stefani et al. [31]. It was shown that, in HeLa cells, while downregulation of MCU results in a marginal increase in bulk cytosolic Ca^{2+} rises, as induced by IP_3-dependent ER Ca^{2+} release, overexpression of MCU drastically reduced the peak amplitude of the cytosolic Ca^{2+} responses.

The above issues concern primarily Ca^{2+} uptake via the MCU, but clearly the net mitochondrial Ca^{2+} accumulation in the matrix is critically dependent also on the activity of the efflux mechanisms. The importance of the Ca^{2+} efflux pathway in modulating the physiological role of mitochondria in buffering bulk cytosolic Ca^{2+} rises has been much less investigated and almost exclusively based on the use of benzothiazepine drugs, i.e., diltiazem, clonazepam, and CGP-37157. All these drugs, however, exhibit different cell effects: diltiazem can also inhibit L-type Ca^{2+} channels [64]; clonazepam does not significantly alter cytosolic Ca^{2+} transients up to 100 μM, but at higher concentrations, it can affect SERCA and sarcolemmal Na^+/K^+ ATPase [64]; CGP-37157 has been reported to inhibit SERCA and to activate RyR1, as well as L-type Ca^{2+} channels [60, 75].

With this limitation in mind, it has been shown that addition of CGP-37157 or clonazepam can substantially increase the intramitochondrial Ca^{2+} peaks in cells whose mitochondria are impaired in their Ca^{2+} uptake capacity due to mutation in their mitochondrial DNA that affects oxidative phosphorylation [12]. In normal cells, on the contrary, addition of CGP-37157 is expected mainly to retard the decay of a mitochondrial Ca^{2+} peak and possibly to increase the peak amplitude of the mitochondrial Ca^{2+} rise. Indeed, in a number of cell lines (e.g., HeLa, COS, etc.) and primary cultures (e.g., brain cells, adrenal medulla, and pancreatic islets), CGP-37157 has been found to decrease the speed of mitochondrial matrix Ca^{2+} decay, while the effect on the Ca^{2+} peak rise was generally quite modest [12]. Of interest, Ishii et al. showed that, during agonist-stimulated cytoplasmic Ca^{2+} oscillations, the efflux of Ca^{2+} from mitochondria plays an essential role in favoring not only the fast refilling of the ER but also, by increasing the local $[Ca^{2+}]$ close to the IP_3 receptors, the reactivation of these channels and the onset of another cycle of Ca^{2+} release from the ER [55]. Under these conditions, CGP-37157 blocks the regenerative Ca^{2+} oscillations. The authors thus suggest that the Na^+/Ca^{2+} exchanger of mitochondria may have a pacemaker role in the generation of cytosolic Ca^{2+} oscillations.

More complex is the situation regarding striated muscles. In mature skeletal muscle in vivo, addition of CGP-37157 slows the decay of the mitochondrial Ca^{2+} peaks during single twitches [101]. Upon repetitive short tetanic stimulations, addition of CGP-37157 caused a substantial increase of basal matrix Ca^{2+}, indicating that inhibition of the Ca^{2+}/Na^+ exchanger results in a net Ca^{2+} accumulation by the organelles that may eventually lead to a Ca^{2+} overload [101]. As to the effects on cytoplasmic Ca^{2+} changes, inhibition of the Na^+/Ca^{2+} exchangers (by increasing the Ca^{2+} uptake by mitochondria) may augment their Ca^{2+} buffering capacity and thus reduce the amplitude of the cytosolic Ca^{2+} peaks. This effect has been studied in particular in cardiac myocytes. Indeed, Maack et al. [63] found that in isolated mature cadiomyocytes, CGP-37157 potentiated the diastolic mitochondrial Ca^{2+} increase and reduced the amplitude of the systolic Ca^{2+} transient in the cytoplasm. Confirming a potential role of mitochondria in buffering the cytoplasmic Ca^{2+} peaks in cardiac cells, the same authors found that Ru360 dialyzed into the cell inhibited mitochondrial Ca^{2+} uptake, but enhanced the amplitude of cytosolic Ca^{2+} transients [63]. Along the same lines, in experiments where cytosolic and mitochondrial Ca^{2+} were measured in parallel, Bell et al. [6] confirmed that clonazepam increased mitochondrial Ca^{2+} rises, while the peak of systolic Ca^{2+} transient in the cytosol was reduced. Other groups, however, obtained different results using similar approaches (for a review, see [47]). These and similar experiments confirm that the results obtained with pharmacological tools, i.e., drugs acting on NCLX or MCU, must be taken with caution and further confirm the need for a more direct genetic approach to alter the level of the exchangers to better understand their role in the physiology of the cell.

Mitochondrial Ca^{2+} and ATP synthesis

This is an extensively reviewed field, and the reader is referred to a few recent papers for details [33, 46]. Unlike the other aspects of the functional role of mitochondrial Ca^{2+} in cell physiology, a general consensus exists concerning the stimulatory role of mitochondrial Ca^{2+} uptake on the activity of the three key enzymes that feed electrons into the respiratory chain (at the level of complex I), i.e., pyruvate dehydrogenase (PDH), isocitrate dehydrogenase (ICDH), and oxoglutarate (or α-ketoglutarate) dehydrogenase (OGDH). All three enzymes increase their V_{max} upon Ca^{2+} binding, though through different mechanisms. In the case of PDH, Ca^{2+} activates a phosphatase associated with the enzyme complex, and the dephosphorylated PDH is more active; in the case of ICDH and OGDH, Ca^{2+} binds directly to the enzymes and

increases their affinity for the substrates [102]. The three enzymes have different Ca^{2+} requirements for activation in vitro, i.e., PDH is half maximally activated at Ca^{2+} concentration of 0.5–1 μM, ICDH at ~20–30 μM, and OGDH at ~1 μM [102]. Evidence for the activation of these enzymes by intramitochondrial Ca^{2+} goes back to the mid 1970s [34], and this elegant model is now widely accepted. Indeed, direct or indirect evidence of a mitochondrial Ca^{2+}-dependent dehydrogenase activity has been obtained not only in cell cultures but also in vivo. For example, McCormack and colleagues found that activation of PDH occurred in whole hearts upon ionotropic stimulation [66, 67], and that this activation was prevented by RR [68, 112].

Activation of the dehydrogenases has been invariably associated with the idea that this should result in activation of electron flow in the respiratory chain and, as a consequence, increased ATP synthesis. Indeed, rises in cytoplasmic and intramitochondrial ATP have been observed following a rise in mitochondrial Ca^{2+} in different model systems, from cell lines to skeletal muscle myotubes to β pancreatic cells (and, under special conditions, in heart cells). Most importantly, buffering cytoplasmic Ca^{2+} increases with a cytosolic Ca^{2+} chelator (and thus inhibiting mitochondrial matrix Ca^{2+} increases) or buffering the intramitochondrial Ca^{2+} rise (by targeting to the matrix a recombinant Ca^{2+} binding protein) results in inhibition of the ATP increase induced by stimulation [116]. Moreover, in several cell types, it has been observed that a rise in mitochondrial Ca^{2+} results in a net increase of mitochondrial NADH reduction, as expected by activation of the dehydrogenases (for a review, see [99]). More recently, Foskett and coworkers demonstrated that even in "resting" cells a continuous fast transfer of Ca^{2+} from the ER to mitochondria is essential to maintain an optimal activity of mitochondrial ATP synthesis [19]. Indeed, blocking this Ca^{2+} transfer (for example, by downregulating the IP_3 receptors) results in a dramatic activation of autophagy. Although the model is largely accepted, few points still need to be resolved. In particular, it is unclear whether the rate-limiting step in electron flow in mitochondria actively synthesizing ATP (as in living cells) is the feeding of electrons into complex I [69], the ATP/ADP ratio [21], or the ATPase (Fig. 3). If the latter were the case, an increase in the activity of the enzyme would be necessary in order to increase the synthesis of ATP. Several studies have indeed suggested that Ca^{2+} may regulate the ATP synthase [54, 117], but direct proof in living cells of this phenomenon is still missing. An interesting result has been recently obtained by Manfredi and coworkers [1, 2]. These authors demonstrated that the activity of cytochrome *c* oxidase can be increased by intramitochondrial cAMP-dependent phosphorylation (possibly mediated by matrix-located PKA). In turn, intramitochondrial cAMP is controlled by a matrix-located adenylate cyclase sensitive to HCO_3 and Ca^{2+}.

It should be stressed that other enzymes directly or indirectly involved in the electron flow through the respiratory chain are activated by Ca^{2+}, in particular

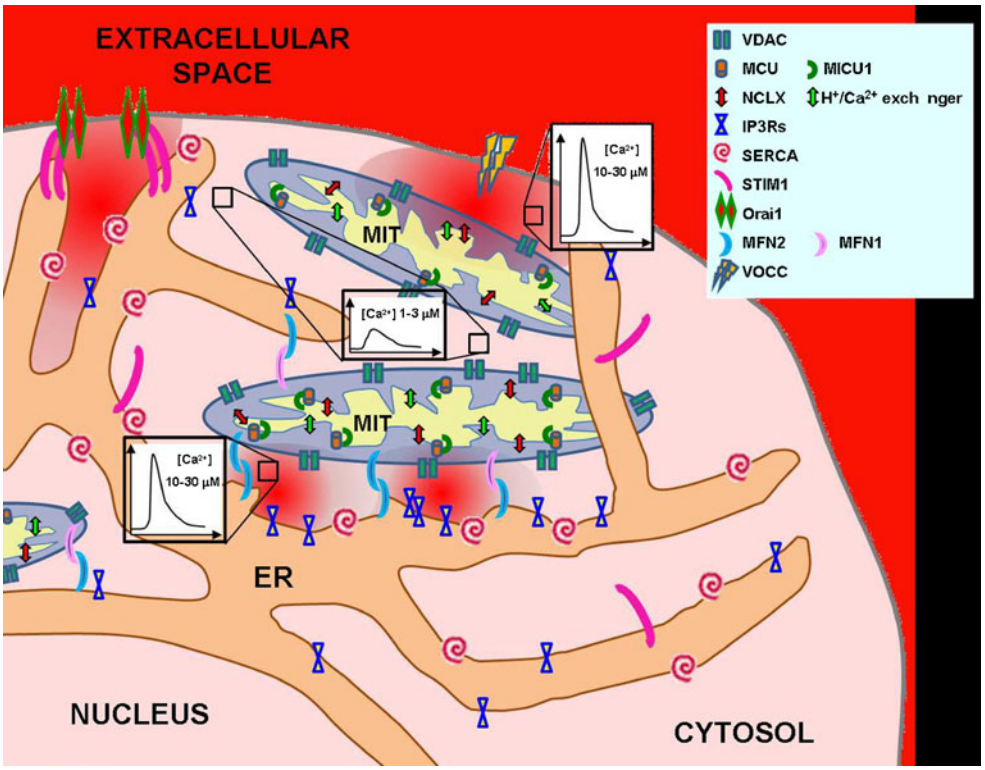

Fig. 3 Schematic model of Ca^{2+} handling by mitochondria localized in different cell compartments. Upon Ca^{2+} release from the ER (via IP_3Rs activation) or Ca^{2+} influx from the extracellular space through voltage-operated Ca^{2+} channels (VOCCs), Ca^{2+} hot spots are generated in the proximity of mitochondria. We proposed (see text and [43]) that mitochondria cannot get close enough to the Orai1 channel mouth due to the presence of ER cisternae physically interacting with the plasma membrane

two mitochondrial substrate carriers, aralar and citrin [25], and the enzyme glycerol phosphate dehydrogenase [51]. The first two are isoforms of the mitochondrial aspartate/glutamate carrier and thus play a role in the accumulation of oxidizable substrates into the matrix; glycerol phosphate dehydrogenase, on the contrary, can feed electrons directly at complex II of the respiratory chain. This enzyme is particularly active in insect flight muscle, and it is also a component of the redox shuttle whereby some mammalian tissues oxidize cytosolic NADH. In these cases, however, the Ca^{2+}-binding site is located on the outer surface of the inner mitochondrial membrane and, accordingly, the Ca^{2+} activation depends on the amplitude and kinetics of the Ca^{2+} increases in the intermembrane space.

Conclusions

Mitochondria are highly dynamic cellular organelles with multiple functions in cell physiology: they represent the powerhouse of eukaryotic cells, play an essential role in metabolism, and control the intrinsic pathway of programmed cell death. In this chapter, we have concentrated our attention on the general principles that govern mitochondrial Ca^{2+} homeostasis and its specific characteristics in different tissues. In the last few years, the molecular identity of key components of the molecular Ca^{2+} homeostatic toolkit has been unraveled, thus offering the possibility of addressing with novel and specific genetic tools the many open questions concerned with the physiological role of these organelles in shaping the amplitude and spatial pattern of Ca^{2+}-dependent signaling. Most relevant, we are only beginning to understand the role of mitochondrial Ca^{2+} in human pathologies, from neurodegenerative diseases to metabolic syndrome, to cancer, and it is easy to predict that this field will continue to represent a hot topic of research in the years to come.

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