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Regulation of Ca^{2+} exchanges and signaling in mitochondria

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Abstract

Mitochondrial calcium ($_mCa^{2+}$) homeostasis also plays a key role in the buffering of cytosolic calcium ($_cCa^{2+}$) and calcium transported into the mitochondrial matrix regulates cellular metabolism, migration and cell fate decisions. Recent work has highlighted the importance of mCa^{2+} homeostasis in regulating cellular function. The discovery of the mCa^{2+} uptake complex has shed new light on the role of $_mCa^{2+}$ dynamics in cytoskeletal remodeling, mitochondrial shape and motility in cellular dynamics. Here we attempt to decipher the vast landscape of calcium regulatory effects of the mitochondria, the underlying mechanisms and the dynamics that control cellular function.

Introduction

Spatio-temporal regulation of cellular signals by the calcium ion (Ca^{2+}) as a versatile second messenger is essential for a variety of kinetically different cellular processes from fertilization to cell death [1]. Temporally, short processes like endocytosis and long processes such as gene transcription are regulated by Ca^{2+}. Intracellular Ca^{2+} signals are regulated by Ca^{2+} influx through the plasma membrane (PM) (extracellular [Ca^{2+}] ~1 mM) [2] and Ca^{2+} release from intracellular stores, in particular from the Golgi ([Ca^{2+}] ~300 μM) [3] and the ER ([Ca^{2+}] ~200–650 μM [4]). Plasma membrane channels or release from intracellular Ca^{2+} stores cause a transient Cytosolic Ca^{2+}(cCa^{2+}) rise [5]. The main path for Ca^{2+} entry in non-excitable cells is (inositol 1,4,5-trisphosphate) IP_3-induced ER store release and store-operated Ca^{2+} entry (SOCE), allowing extracellular Ca^{2+} entry into the cell through plasma membrane localized Ca^{2+}-activated Ca^{2+}-release channels, made up of ORAI1/2/3 protein subunits and gated by interactions with STIM1/2, or TRPC (TRPC1–6).

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Putney et al proposed capacitive calcium entry as a mechanism for receptor-regulated Ca^{2+} entry [5]. At any given point, resting $cCa^{2+}$is kept low (below 100 nM), and this is achieved by Ca^{2+} efflux action of pumps, exchangers and organelles such as ER and the mitochondria, which act as Ca^{2+} sinks. To maintain the optimal cCa^{2+} concentration ([Ca^{2+}] ~100nM), intracellular Ca^{2+} stores are constantly refilled while cCa^{2+} is extruded from the cell by the plasma membrane Ca^{2+} ATPase (PMCA) pump, exchangers and solute carriers. Intracellular Ca^{2+} is mainly stored in the ER lumen, which is constantly refilled by the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump [5]. Upon cell stimulation, cell surface receptors can activate phospholipase C (PLC), which hydrolyses the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) to form inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). Subsequently, IP3 diffuses to the ER membrane, where it binds the IP3 receptor (IP3R), triggering Ca^{2+} release from the ER. ER Ca^{2+} can be released directly into the cytosol or into organelles such as mitochondria [6], in turn contributing to cellular Ca^{2+} homeostasis.

cCa^{2+} transients are defined and shaped by the mitochondria. The first observation of mitochondrial Ca^{2+} ($_{m}Ca^{2+}$) uptake was evidenced five decades ago when several groups witnessed isolated mitochondria to buffer Ca^{2+} [5]. Since then, mCa^{2+} has been found to be utilized by the dehydrogenases of the TCA cycle (pyruvate dehydrogenase, iso-citrate dehydrogenase and α-ketoglutarate dehydrogenase) for ATP generation, to shape cCa^{2+}dynamics and to activate various cell death pathways [7]. Mitochondrial calcium uniporter (MCU), situated on the inner mitochondrial membrane (IMM), enables uptake of substantial amounts of Ca^{2+} from the cytosol during intracellular Ca^{2+} signaling events [8]. The selectivity of MCU to Ca^{2+} has been well established [9][10]. Although the affinity of MCU for Ca^{2+} is very high [equilibrium dissociation constant (Kd) ≈ 2 nM], MCU relies on the highly negative membrane potential of the IMM to drive Ca^{2+} into the matrix, as the basal Ca^{2+} concentration in the cytosol is not sufficient to allow an efficient mitochondrial uptake through the MCU [8]. Nevertheless, cytosolic Ca^{2+}concentrations above a set threshold are needed to potentiate MCU activity (Figure 1).

It is now well established that one of the main functions of mitochondria-ER contact sites [11], stabilized by tethering proteins like mitofusin 2 (MFN2) [12], is to generate highly localized and concentrated Ca^{2+} microdomains, which facilitate Ca^{2+} transport into mitochondria [5]. When released from the ER, Ca^{2+} first passes from the cytosol to the intermembrane space (IMS) through the outer mitochondrial membrane (OMM). Passage through the OMM occurs through the voltage-dependent anion channel (VDAC); from the IMS, MCU [13] subsequently transports Ca^{2+} across the IMM into the matrix. Mitochondrial Ca^{2+} uptake is involved in control of metabolism and ATP production to the regulation of cell death, conversely depletion of mitochondrial membrane potential abrogates mitochondrial Ca^{2+} uptake, and defects in the respiratory chain have been associated with a decreased ability of mitochondria to uptake Ca^{2+} [14][15]. Mitochondrial Ca^{2+} uptake has also been linked to numerous physiological functions such as cell death, autophagy, skeletal muscle trophism, immunity, cardiomyocyte contraction and heart rate [14][15]. In the last six years, the discovery of MCU and its regulators have allowed us to obtain a better understanding of the mechanisms underlying mitochondrial Ca^{2+} regulation and to investigate the role of MCU function in intracellular Ca^{2+} signaling.

ER-Mitochondrial contact sites: Ca^{2+} homeostasis between membranes

Precise maintenance of compartmentalized levels of Ca^{2+} maintain physiologic intracellular ionized calcium is essential to calcium homeostasis. Changing mitochondrial motility mediates intracellular proximity to various cellular organelles or the plasma membrane to facilitate Ca^{2+} homeostasis. The specific mechanism of cellular Ca^{2+} homeostasis occurs by the exchange of Ca^{2+} between the ER and mitochondria. Inter-organelle communication such as structural and functional interactions between ER and mitochondria play a central role in multiple pathways, ranging from cellular processes or signaling pathways, all finally impacting cellular metabolism. Ca^{2+} transfer into mitochondria occurs at highly specialized regions of close contacts between mitochondria and ER called mitochondria associated membranes (MAMs). Transfer of Ca^{2+} from ER to mitochondria is crucial for the control of mitochondria energy metabolism, since mitochondrial Ca^{2+} levels control the activity of Krebs cycle's dehydrogenases and impact ATP synthesis [16]. Ca^{2+} release into the cytosol through the ER Ca^{2+} release channel IP3R creates a cCa^{2+} nanodomain that is sufficiently concentrated, within the μM range, to be sensed by adjacent mitochondria [17]. A complex comprising of IP3R, VDAC at the mitochondrial OMM and chaperone glucose-regulated protein 75 (GRP75) has been shown to regulate ER-mitochondrial Ca^{2+} transfer [18]. The ER protein sigma receptor-1 (SigR1) also forms a complex with IP3R3, and upon ER Ca^{2+} depletion, Sig1R binds IP3R3 and stabilizes it to prolong Ca^{2+} signaling from the ER to mitochondria via IP3R at MAMs [19]. Increasing Sig1R expression in cells counteracts ER stress, thus inhibiting apoptosis [19].

Recent reports demonstrate that mitochondrial calcium uptake 2 (MICU2), a gatekeeping regulator of MCU, controls the cross talk between IP3R and MCU [20]. While the MAM tethers Mdm1p, Mdm10p, Mdm34p and Mdm12p have been identified, which make up the ER-mitochondria encounter structure (ERMES) [19]. Nevertheless, the ER vesicle-associated membrane protein-associated protein B (VAPB) and the mitochondrial membrane protein tyrosine phosphatase interacting protein-51 (PTPIP51) proteins have been shown to form such a tether in mammalian cells, which when disrupted influences mitochondrial Ca^{2+} uptake [21]. During early phases of ER stress, ER mitochondria contacts are brought closer by ~5 nm, and this coupling has been shown to promote mitochondrial respiration and bioenergetics [22]. Further, sustained activation of ER stress impairs mitochondrial metabolism [23], suggesting a strong link between metabolic insufficiency and ER stress-mediated apoptosis [17]. In addition, genetic or pharmacological inhibition of IP3R alters mitochondrial function, lowering ATP production and triggering autophagy [24]. Moreover, ER stress has been linked to the loss of MAM proteins such as phosphofurin acidic cluster sorting protein 2 (PACS2) [25], SigR1, mitofusin 2 (Mfn2) or cyclophilin D (CypD) [19]. Conversely, while early phases of ER stress increased ER-mitochondria coupling to promote mitochondrial respiration and bioenergetics (as mentioned above), massive and/or prolonged mitochondrial Ca^{2+} accumulation induced swelling and dysfunction of the organelles [23].

Protein kinase-like endoplasmic reticulum kinase (PERK), an important ER stress sensor, is localized at MAMs and increases the physical coupling between both organelles [26]. Furthermore, PERK activity is regulated by Mfn2 [27], an important tether at the MAM interface [12], which has been associated with ER stress responses [19]. As unfolded protein

response (UPR) signaling controls insulin action [28] and secretion [29], MAMs could therefore regulate glucose homeostasis through effects on UPR signaling. At MAMs, CDSH iron sulfur domain protein 2(Cisd2) interacts with GTPase, IMAP Family Member 5 (Gimap5) and modulates mitochondrial Ca^{2+} uptake, in order to maintain intracellular Ca^{2+} homeostasis [30]. Interestingly, conventional loss of Cisd2 altered mitochondria function, impaired glucose tolerance and induced premature aging, whereas white adipose tissue(WAT)-specific loss of Cisd2 impaired the Ca^{2+} buffering capability of mitochondria, increased $_{c}Ca^{2+}$, impaired the differentiation of WAT, and decreased insulin-stimulated glucose transport [30]. Furthermore, $[Ca^{2+}]_c$ increases have been shown to slow/stop mitochondrial motility via Ca^{2+}-sensitive uncoupling of the connection with the microtubules, in which the mitochondrial rho GTPase-1 (MIRO1) plays a central role [31]. Ca^{2+}-sensitive Miro1/2 promote engagement between ER Ca^{2+} release nanodomains and mitochondria [32]. Colocalization of MIRO1 with ER-mitochondria contacts has been reported in mammalian cells, and the yeast homologue of MIRO1, Gem1, has been shown to be a regulatory component of the ERMES complex, supporting a role for MIRO/Gem1 in the ER-mitochondrial contact formation [32].

Mitochondrial Ca^{2+} uniporter

Mitochondrial calcium dynamics began to be appreciated with the discovery of the Ruthenium Red sensitive mitochondrial channel [8,33]. Attempts were made to identify the structure and composition of this membrane-potential-dependent core component responsible for Ca^{2+} transport into the mitochondrial matrix. Those efforts were successful, as mitochondrially localized Ruthenium Red sensitive CCDC109A with two putative transmembrane domains was identified and named MCU [34][8][35] (Figure 1). Voltage clamp experiments revealed that inwardly rectifying Ca^{2+} currents were mediated by MCU, which matched the properties characterized in the Kirichok and co-worker seminal work[36]. The MCU C-terminal domain (CTD) is composed of two transmembrane TM domains (TM1 and TM2) and two coiled coil domains required for interaction with several regulators (see below). An initial NMR-driven model of the *Caenorhabditis elegans* MCU CTD suggested the loop connecting TM1 and TM2 contains conserved Asp240 and Glu243 residues (*i.e.* Asp261 and Glu264 in humans), important in the Ca^{2+} selectivity by MCU [37]. While the Asp240 and Glu243 are, indeed, vital for Ca^{2+} selectivity, these acidic residues are likely integrated into TM2 based on more recent structural investigations of full-length MCU from various organisms (Figure 2A and 2B). Similarly, while the *C. elegans* MCU CTD structure suggested a pentameric assembly [37], four independent groups have firmly established a tetrameric architecture for fulllength MCU through several cryo-electron microscopy and crystal structures [9,10,38–41]. These structures show the conserved W-D-Φ-Φ-E-P-V-T-Y sequence motif of the MCU pore (where and D and E are Asp261 and Glu264 in human MCU, respectively), forms the Ca^{2+} selectivity filter by assembling to two acidic rings separated by one helical turn in TM2 along the central axis of the channel pore [38]. Further, the Asp and Glu sidechains of the conserved W-D-Φ-Φ-E-P-V-T-Y motif (also known as the DIME motif) are pointed towards the central axis [39] (Figure 2B).

The structure of N-terminal domain (NTD) of MCU, encoded by exons 3 and 4 of the *MCU* gene, adopts a β-grasp like fold comprised of two α−helices and six β-strands; moreover, this central core also contains two highly conserved leucine rich loops [42][43,44]. Atomic resolution structures of MCU-NTD revealed a cluster of negatively charged residues called the MCU-regulating acidic patch (MRAP) on the surface of the β-grasp fold, which binds divalent cations. Remarkably, interaction of either Ca^{2+} or Mg^{2+} with the MRAP causes a loss of mCa^{2+}uptake in mammalian cells, highlighting a mechanism for matrix divalent cation-mediated regulation of MCU channel function. The sensitivity of MCU function to MCU-NTD was reinforced by data showing that overexpression of MCU with MRAP mutants could also inhibit the channel [44].

Positive and negative regulators of MCU

Interestingly, reconstitution of the MCU complex in yeast, showed that expression of the human MCU alone was not sufficient to promote mitochondrial Ca^{2+} uptake [45]. MCU regulator 1 (MCUR1) is a positive regulator of MCU function. MCUR1 was identified in an RNAi screen of mitochondrial proteins where knockdown resulted in the inhibition of mitochondrial Ca^{2+} uptake. The IMM localization of MCUR1 was verified experimentally for the yeast FMP32 and human paralogs of MCUR1 (initial identified as CCDC90B) [42] [46]. MCUR1 directly interacts with MCU to promote Ca^{2+}-uptake; thus, loss of MCUR1 results in a reduced mCa^{2+} uptake and has been shown to perturb bioenergetics [47,48]. MCUR1 is also an assembly factor for complex IV cytochrome oxidase (COX), and loss of MCUR1 results in impaired oxidative phosphorylation (Ox-phos), leading to reduced IMM potential [49]. Germ line deletion of MCUR1 did not show any discernable phenotype, while cardiac specific MCUR1 knockout (KO) was postnatally fatal at ~3 weeks [42]. Electrophysiological and biochemical studies from tissue specific KOs demonstrated a significant decrease in MCU current due to the disruption of the MCU super-complex in the absence of MCUR1. Our group showed that MCU failed to assemble as a higher order complex in the absence of MCUR1, which also has transmembrane and coiled-coil domains. The coiled-coils, which are well known protein-protein interaction domains, and the perturbation of MCU assembly in the absence of MCUR1 suggest MCUR1 has, at least in part, a scaffolding function [50]. Furthermore, we and others found that the MCU N terminal region is likely to associate with MCUR1 rather than MICU1 or MICU2. Consistent with a scaffolding role, recent work by Adlakha and colleagues demonstrated MCUR1 directly interacts with MCU, which is destabilized upon Ca^{2+} binding. Thus, Ca^{2+} could regulate the MCUR1:MCU interactions. Further work needs to be performed to elucidate and characterize the exact mechanisms underlying MCUR1-mediated regulation of MCU [51].

EMRE is a 10-kilodalton, metazoan-specific single transmembrane protein with an aspartate rich C-terminus. EMRE maintains the pore in an open conformation while additionally transducing MICU1/2 Ca^{2+} sensing to pore gating [45][40]. EMRE interacts directly with MCU through a GXXXG motif in the transmembrane helix, bridging the Ca^{2+}-sensing role of MICU1 and MICU2 with the Ca^{2+}-conducting role of MCU [42]. Disrupting the EMRE–MICU1 interaction leads to loss of MICU1–MICU2 from the uniporter complex, yielding a MCU–EMRE subcomplex with a high open probability [52].

The IMS localized, EF hand-containing protein mitochondrial Ca^{2+} uptake-1 (MICU1) was identified well before the discovery of MCU. MICU1 belongs to a family of MICU regulators that also includes MICU2 and MICU3 [53]. Our data proposed MICU1 to be the MCU "gatekeeper" [42,48][1], sensing a rise in $_{c}Ca^{2+}$ and increasing the open probability of MCU [42,54]. Ca^{2+}-free conditions reveal MICU1 to form a hexamer that directly or indirectly interacts with MCU and inhibits its activity through the C-terminal helix [55]. Like most ion channels that open upon agonist stimulation, the MCU channel open probability increases when $_{c}Ca^{2+}$ rises above 2 μM [42,48]. MICU1-MICU2 hetero-dimers co-operatively function as a switch for MCU-mediated $_{m}Ca^{2+}$ uptake [56]. Disulfide bonded MICU1 and MICU2 or MICU3 at low $_{c}Ca^{2+}$ has been proposed to inhibit MCU from conducting Ca^{2+}. $_{c}Ca^{2+}$ increases are sensed by the MICU EF hands, which undergo rearrangements and promote Ca^{2+} influx through the MCU complex [42,57] Figure 1.

MCU is also negatively regulated by a dominant negative form of MCU, MCUb, whose tissue expression profile differs from MCU [58]. MCU interacts with MCUb; however, despite showing a high sequence identity and similarity with MCU, MCUb alone does not reconstitute Ca^{2+} channel activity. There is evidence to suggest that other paralogs of MCU do not act as dominant negative effectors of the channel. In the MCU complex of trypanosomatids, two additional unique paralog subunits (*i.e.* MCUc and MCUd) are required for Ca^{2+} transport [59]. Ultimately, hetero-oligomeric structures are a likely result of the heterologous expression and assembly of MCU, MCUb, MCUc, and MCUd.

Structural mechanisms of MCU regulation within super-complexes.

Interestingly, in the context of full-length MCU tetramers, the NTD region adopts different assembly patterns depending on the MCU orthologue. For example, the MCUNTD from lower order organisms assembles as a dimer of dimers directly under the pore [9,10,38,39], but a dimer of tetramers across two human MCU channels[41]. In other words, the mode of NTD assembly is distinct in MCU proteins from different species. MCU-NTD is arranged in a compact circle in fungal structures [9,10,38,39], while zebrafish and human structures (*i.e.* metazoan) show more linear, semi-circular tetrameric assemblies [9,10,38,39,44]. In the human MCU structure, MCU-NTD tetramers from different channels interact, dimerizing entire channels (Figure 2A&2B). Co-complexation with EMRE is key to this full channel dimerization, since elimination of EMRE results in movement of the NTD semi-circular assembly from the side of the channel to the base, precluding channel-channel interactions[44]. It has been suggested that full channel dimerization via the NTDs could help to preferentially locate MCU to the curved outer regions of the IMM (closest to the OMM), where channel complexes would be well-situated to most directly respond to μM increases in Ca^{2+} [44].

The recent human EMRE-MCU co-complex structure has also revealed an equimolar stoichiometric assembly between these proteins within each channel. Mechanistically, the single EMRE transmembrane domain makes extensive contacts with the MCU TM1 and TM2 regions of different subunits, and the EMRE N-terminal region interacts with coiled-coil-2 of MCU, located on the matrix side of the channel. This coiled-coil interaction is believed to pull the juxtamembrane loop of MCU away from the pore exit, allowing Ca^{2+}

permeation [44] (Figure 2C). It is important to note that the required stoichiometry for a functional channel may not be 4 EMRE:4 MCU subunits, as recent work using MCU-EMRE concatemers have shown that 1 EMRE per 4 MCU subunits is sufficient for channel activation, while 2 EMRE:4 MCU subunits recapitulates more closely the size and activity of endogenous channels[60]. In fact, the same study demonstrated that a 4:4 EMRE-MCU stoichiometry increased the threshold of cytoplasmic cCa^{2+} necessary for channel activation.

A study recently submitted to bioRxiv resolved a human MCU-EMRE-MICU1MICU2 super-complex structure by cryo-EM [61]. A very similar dimer of tetrameric MCU channels was elucidated as shown in Figure 2A, with 4 EMRE subunits integrated into each MCU channel (*i.e.* 4 EMRE: 4 MCU subunits per one channel). However, in this super-complex, one MICU1 and one MICU2 subunit was also complexed per channel, showing an overall EMRE:MCU:MICU1:MICU2 stoichiometry of 4:4:1:1 per channel. Lipid (*i.e.* cardiolipin and phosphatidylcholine) interactions with protein components were also found to play a role in super-complex stabilization [61]. Interestingly, in this super-complex structure, MICU1-MICU2 heterodimers were found to interact across individual channels, away from the pore openings, much like the MCU NTD interactions observed across channels that stabilize the matrix side of the channel. It is also noteworthy that the non-occluding position of MICU1-MICU2 relative to the pore is consistent with a bioRxiv submission showing that Ca^{2+} binding to the MICUs increases the open probability of MCU pore, which was found to be a constitutively active Ca^{2+} channel [54]. The mechanism underlying the open probability regulation by the MICUs may involve the interactions between the poly-basic region of MICU1 [54] and aspartate rich C-terminal region of EMRE, identified in the cryo-EM structure [61] and previously reported. EMRE may also play a role in bridging MICU1-MICU2 Ca^{2+} sensing in the IMS with matrix Ca^{2+} and Mg^{2+} sensing by MCU, as a recent submission to bioRxiv has *i*) verified that both Mg^{2+} and Ca^{2+} binding to the MCU-NTD MRAP can feedback inhibit the channel, as we first demonstrated ([44], and *ii*) suggested that divalent cation occupancy of MRAP determines the efficacy of MICU1-MICU2-mediated regulation of MCU channel activity [62].

Ca^{2+} exchangers regulate mitochondrial Ca^{2+} efflux

Calcium efflux from the mitochondria is believed to be carried out by Ca^{2+}/hydrogen (H^+) and a sodium (Na^+)/Ca^{2+} exchanger function. The mitochondrial Na^+/Ca^{2+}/lithium (Li^+) exchanger (NCLX) is an IMM protein that extrudes one Ca^{2+} from the mitochondria for every three Na^+ or Li^+ imported; NCLX has an estimated turnover rate of 1,000 cycles per second [17][63]. NCLX is regulated by PTEN-induced putative kinase 1 (PINK1) and PKA, where PINK1 causes reduced mitochondrial Ca^{2+} efflux, and PKA activation in the absence of PINK1 restores efflux [64]. NCLX regulates SOCE indirectly through Ca^{2+} overload induced ROS [65]. Inhibition of NCLX in primary pancreatic β-cells inhibited mitochondrial Ca^{2+} efflux induced by high glucose, coinciding with reduced rates and amplitudes of $_cCa^{2+}$ signals and delayed insulin secretion [17]. Thus, NCLX plays a critical role in the communication between the plasma membrane and mitochondria and in regulating $_cCa^{2+}$ signals [66].

The Na^+/H^+ exchanger (NHE), which is responsible for Na^+ efflux from mitochondria into the cytosol, is a major regulator of mitochondrial Na^+ in several cell types [67]. NHE is, therefore, critical for maintaining Na^+ gradients across the mitochondrial membrane, and its function is vital for NCLX activity and for the maintenance of Ca^{2+} homeostasis in mitochondria. Although leucine-zipper EF-hand containing transmembrane protein-1 (LETM1) was originally proposed to be a potassium $(K^+)/H^+$ exchanger on the IMM, several publications have since characterized the mitochondrial Ca^{2+}/H^+ antiporter of this transmembrane protein, both reconstituted in liposomes without any other regulatory proteins and in mammalian cells [68][69][70].

Cytosolic Ca^{2+} drives mitochondrial dynamics and shape

Mitochondria dynamics include motility including long distance and shorter distance along microtubules, ensured by mitochondrial adaptor proteins, motor proteins and cytoskeleton components, which are regulated by $_cCa^{2+}$. The canonical opinion in the field is that mitochondria undergo changes in shape in response to insults causing mitochondrial fragmentation, and smaller fragmented mitochondria undergo rapid phenotypic changes when compared to massively elongated mitochondria. Drp1 dependent fragmentation of the mitochondria integrates Ca^{2+} signals to modulate mitochondrial shape in cancer. Drp1 recruitment to mitochondria is regulated by the Ca^{2+} dependent phosphatase calcineurin, dephosphorylating Drp1 at Ser637 upon a rise of cCa^{2+} [71]. However, recent findings show a novel mechanism by which mitochondria transition from long filamentous to short circular morphologies. The OMM bound EF-hand containing Ras GTPase Miro1/2 senses $_cCa^{2+}$ levels to modulate mitochondrial movement locally and globally [46,47]. Calcium sensing protein Miro1 is tethered to the OMM and closely associated with the motor complexes kinesin/track/syntaphilin. We demonstrated Drp1 independent mitochondrial shape transition, termed MiST, due to high cCa^{2+} influx, specifically via Miro1 EF-hand-1 hand sensing of high cCa^{2+}. The Kd for MiST was determined to be > 1 μM cCa^{2+} [73]. This Miro-dependent mechanism could likely be an alternative to Drp1 dependent mitochondrial fragmentation to aid motility in non-excitable as well as excitable cells. In an attempt to elucidate how mitochondrial DNA (mtDNA), located in the matrix of the mitochondria, is released into the cytosol and activates the cGAS STING pathway that causes cells to secrete type 1 interferon, studies showed a dynamic change in mitochondrial phenotype from filamentous to globular upon treatment with Bcl2 inhibitor[74]. Herniation of IMM releases mtDNA through OMM pores formed by Bax-Bak oligomers [74]. Interestingly, there is evidence to suggest basal mitochondrial Ca^{2+} concentration could influence mitochondrial motility [75]. Despite these observations and signaling pathway illuminations, mitochondria shape and dynamics are influenced by various unknown factors and remain to be fully elucidated.

Concluding Remarks and Perspectives

Cellular Ca^{2+} dynamics depends on the precise compartmentation of Ca^{2+} to elicit regulated responses to stimuli. The ER-mitochondrial contacts function to bring about a strict regulation of Ca^{2+} release and uptake into mitochondria upon the signal transduction in a spatio-temporally regulated manner. Recent evidence has shed a light on the crucial role of MCU and its regulators in cellular Ca^{2+} dynamic signaling for metabolic needs as well as

Ca^{2+} homeostasis. Independently of the role of MCU in cancer cell migration, different studies have highlighted the role of mitochondrial Ca^{2+} homeostasis in immune cell polarization and chemotaxis. Taken together, these data obtained in different specialized cells and animal models highlight the crucial and evolutionarily conserved function of MCU. So far, the non-exclusive connections between ATP and ROS production and cCa^{2+} signal regulation have been investigated. To fully elucidate these mechanisms, it will be important not only to decipher the potential role of MCU on Ca^{2+} flicker formation/intensity but also the impact of MCU on mitochondrial motility/shape dynamics that govern Ca^{2+} micro and nanodomains, controlling local and global cellular Ca^{2+} dynamics.

The generation of germline and tissue specific MCU KO animals permitted the understanding of the physiological significance of the channel. Pathological understanding of the MCU channel has been under ongoing investigations and requires better designed, more controlled and new studies to fully elucidate its role in disease manifestation and reveal potential therapeutic interventions [76]. For example, given the work showing that MCU can be regulated through Ca^{2+} and Mg^{2+} binding at the MRAP region in the NTD, studies on supplementing Mg^{2+} to limit MCU channel activity under conditions of Ca^{2+} overload may be warranted. Similarly, studies have shown, like most ion channels, MCU also undergoes oxidative modifications that can profoundly regulate function. Thus, MCU can serve as a matrix ROS sensor, and understanding the molecular mechanisms of MCU oxidation in inflammatory diseases may open previously unappreciated therapeutic avenues [77]. Furthermore, MCU KO has been shown to trigger mitochondrial shape transition (*i.e.* MiST) due to elevated cytosolic Ca^{2+}, leading to MiST-mediated cell death and underscoring the vital crosstalk between cytosolic Ca^{2+} signals and mitochondrial Ca^{2+} uptake [73]. Future studies on MiST mediated pathways need to be explored with objectives focused on pathophysiological significance.

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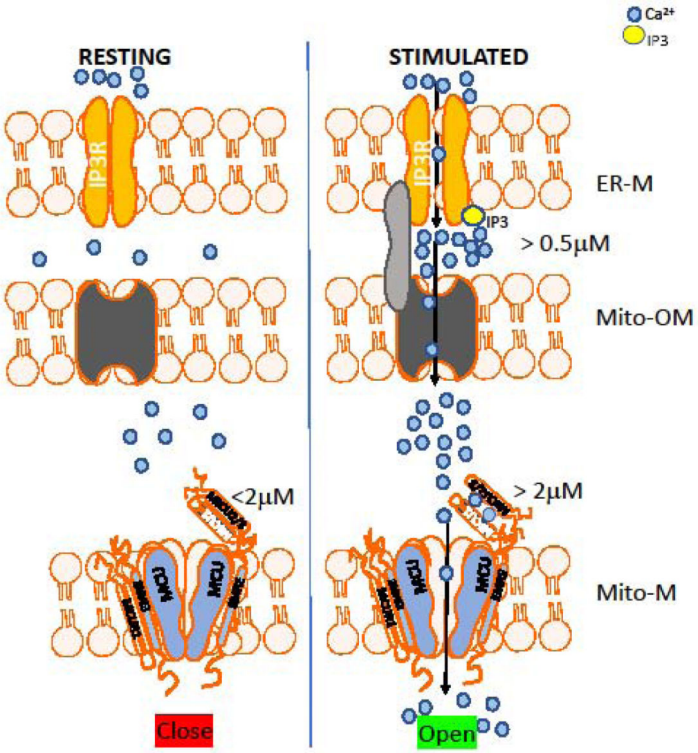

Figure 1. Schematic depicts agonist-induced transfer of calcium from ER to mitochondria.

Resting state refers to the state wherein Ca^{2+} concentration between ER mitochondrial nano domains are <0.5µM and IMM and OMM accumulation of calcium is <2µM. Stimulated state refer to ER-mitochondrial nano domains at >0.5µM after agonist stimulation and post e.g.IP3 association to IP3 receptor resulting in IMM and OMM accumulation of calcium is >2µM. This results in MICU1/MICU2 conformational change controls MCU open Probability through EMRE conformational change.

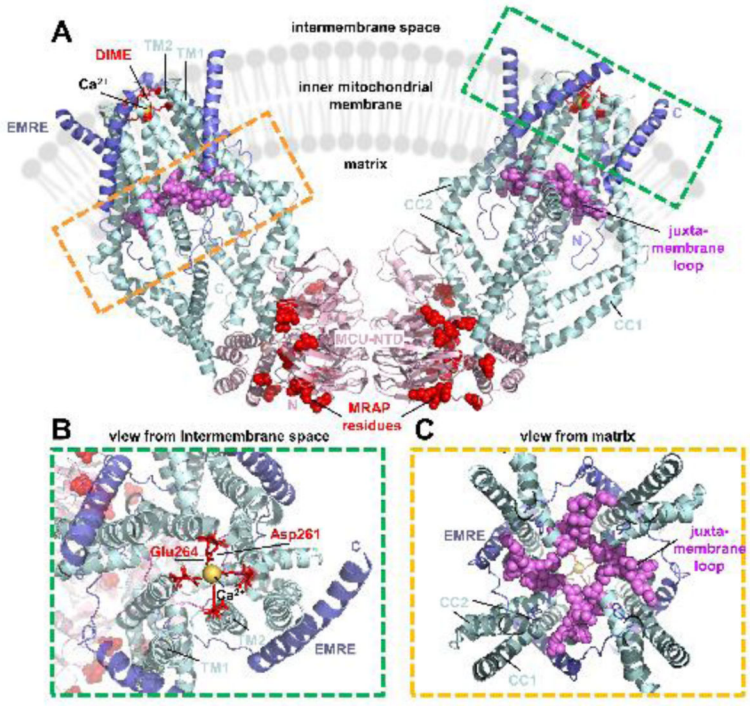

Figure 2. Cryo-EM structure of the human MCU-EMRE super-complex.

A. Dimer of functional MCU tetrameric channels. Each MCU tetramer (cyan) is in complex with four EMRE peptides (blue). The individual MCU channels dimerize via interactions between the N-terminal domains (MCU-NTD; pink). The Asp261 (red sticks) and Glu264 (red sticks) of the DIME motif (indicated) are located closest to the intermembrane space side of each channel. The juxtamembrane loop (magenta spheres) is located on the matrix side of each channel. The residues making up MRAP within each MCU-NTD (red spheres) are localized only between MCU-NTDs within one channel. **B.** Top view (from the intermembrane space) of a single MCU channel highlighting the pore entrance. The Asp261 and Glu264 sidechains (red sticks) of the DIME motif are directed to the centre axis of the channel. Coordination of a single Ca^{2+} (yellow sphere) is primarily mediated by the Glu264 side chain in this structure. The inner surface of the channel is lined with the TM2 helices, while the TM1 helices pack on the outside of each TM2 helix. Four EMRE peptides are oriented at the periphery of each channel. **C.** Bottom view (from the matrix) of a single MCU channel highlighting the pore exit. The juxtamembrane loop (magenta spheres) forms an exit pore constriction. In the presence of EMRE, the juxtamembrane loop does not obstruct the pore exit due to interactions between the N-terminal region of EMRE and CC2 of MCU. In (*A – C*), the MCU and EMRE protein backbone is represented as a cartoon ribbon. TM1/2, transmembrane 1/2 (cyan); CC1/2, coiled-coil-1/2 (cyan); MCU-NTD, MCU Nterminal domain (pink); MRAP, MCU regulating acidic patch (red); Ca^{2+}, calcium ion (yellow); DIME, Asp-Ile-Met-Glu motif; EMRE, essential MCU regulator (blue); MCU, mitochondrial Ca^{2+} uniporter; N, amino terminus; C, carboxyl terminus. The structure images were rendered in PyMOL (Schrodinger, LLC) using the 6O58.pdb coordinates.