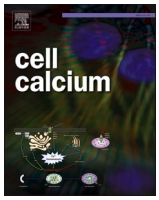
Cell Calcium 112 (2023) 102720

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca



The mitochondrial calcium uniporter complex–A play in five acts

Agnese De Mario [a], Donato D'Angelo [a], Giuseppe Zanotti [a], Anna Raffaello [a,b], Cristina Mammucari [a,b,*]

[a] Department of Biomedical Sciences, University of Padua, 35131, Padua, Italy
[b] Myology Center (CIR-Myo), University of Padua, 35131, Padua, Italy

A B S T R A C T

Mitochondrial Ca^{2+} ($mitCa^{2+}$) uptake controls both intraorganellar and cytosolic functions. Within the organelle, [Ca^{2+}] increases regulate the activity of tricarboxylic acid (TCA) cycle enzymes, thus sustaining oxidative metabolism and ATP production. Reactive oxygen species (ROS) are also generated as side products of oxygen consumption. At the same time, mitochondria act as buffers of cytosolic Ca^{2+} ($cytCa^{2+}$) increases, thus regulating Ca^{2+}-dependent cellular processes. In pathological conditions, $mitCa^{2+}$ overload triggers the opening of the mitochondrial permeability transition pore (mPTP) and the release of apoptotic cofactors. $MitCa^{2+}$ uptake occurs in response of local [Ca^{2+}] increases in sites of proximity between the endoplasmic reticulum (ER) and the mitochondria and is mediated by the mitochondrial Ca^{2+} uniporter (MCU), a highly selective channel of the inner mitochondrial membrane (IMM). Both channel and regulatory subunits form the MCU complex (MCUC). Cryogenic electron microscopy (Cryo-EM) and crystal structures revealed the correct assembly of MCUC and the function of critical residues for the regulation of Ca^{2+} conductance.

1. ACT I. Gather at the IMM: structural and regulatory proteins form the MCU complex

The MCUC is composed of pore forming subunits including MCU, the MCU-dominant negative b (MCUb), the essential MCU regulator (EMRE), and of regulatory subunits including Mitochondrial Calcium Uptake 1 (MICU1), MICU2, MICU3 and MCU regulator 1 (MCUR1).

MCU is a 40 KDa protein located in the IMM [1,2] containing two transmembrane domains (TM1 and TM2). Both the C- and the N-termini of the protein face the mitochondrial matrix [1–3]. The two transmembrane domains are linked by a highly conserved loop that faces the IMS, containing a "DIME" motif of negatively charged amino acids (Asp 260 and Glu 263) that are required for Ca^{2+} permeation [2,4]. Down-regulation of MCU strongly reduces $mitCa^{2+}$ uptake both in living cells, stimulated by Ca^{2+} mobilizing agonists, and in permeabilized cells perfused with buffered Ca^{2+}, without affecting the mitochondrial membrane potential ($\Delta\Psi m$) [1,2]. Coherently, MCU overexpression in cultured cells enhances Ca^{2+} transport in mitochondria evoked by stimulation with IP_3-coupled agonists.

MCUb is a 33 kDa protein encoded by the CCDC109b gene, which shares 50% identity with MCU, has similar topology (with a modified amino acid sequence within the DIME motif), and interacts with MCU, by forming a hetero-oligomer. In living cells, MCUb overexpression reduces the amplitude of $mitCa^{2+}$transients evoked by agonist stimulation, and MCUb silencing elicits the opposite effect, indicating that this protein acts as a dominant-negative subunit of the channel [4]. The expression and stoichiometry of MCU and MCUb vary significantly among mammalian tissues. Some tissues, such as the heart, exhibit a high MCU: MCUb ratio, while others, like skeletal muscle, display a lower ratio, and this represents one of the mechanisms that underlie the different MCU currents recorded in different tissues [5]. MCUb is highly expressed in immune cells, including phagocytes. This is particularly relevant in skeletal muscle regeneration upon damage, where MCUb modulates the conversion of macrophages from the proinflammatory to the pro-resolving phenotype [6].

The Essential MCU Regulator (EMRE) is a 10 kDa protein of the IMM, identified by SILAC-based quantitative mass spectrometry of affinity purified MCU complex [7]. It is composed of a transmembrane domain,

Characters: MCU, Embedded in the IMM, a tetramer of MCU subunits forms the highly selective Ca^{2+} channel.; MCUb, The dominant-negative isoform of MCU preserves mitochondria from excessive Ca^{2+} entry.; EMRE, A single transmembrane domain that, interacting with MCU, is required for channel activity.; MICU1, The first MCUC member to be discovered, it resides in the intermembrane space (IMS) where it senses [Ca^{2+}], thus controlling mitochondrial Ca^{2+} entry.; MICU2, Together with MICU1, it contributes to keep $mitCa^{2+}$ uptake tightly controlled.; MICU3, The third member of the MICU family acts as an enhancer of MCU-dependent mitochondrial Ca^{2+} uptake both in the brain and in the heart.; MICU1.1, Specifically expressed in skeletal muscle, it warrants sustained $mitCa^{2+}$ uptake to meet the needs of a working muscle.; MCUR1, By acting as a scaffolding factor to assemble the MCUC, it directly regulates MCU channel activity.

* Corresponding author.
E-mail address: cristina.mammucari@unipd.it (C. Mammucari).

https://doi.org/10.1016/j.ceca.2023.102720
Received 19 January 2023; Received in revised form 22 March 2023; Accepted 23 March 2023
Available online 24 March 2023

a short N-terminal domain, and a highly conserved acidic C-terminal domain [7]. EMRE is required for MCU activity, as demonstrated by the fact that mitCa^{2+} uptake is abolished in EMRE KO cells. Even though in planar lipid bilayer MCU and the regulatory subunits MICU1 and MICU2 display the ability to interact with each other without the presence of EMRE [8], EMRE has been proposed to play a fundamental role in the interaction between the pore core subunits and the regulatory subunits [7]. Moreover, in yeast cells, that lack mitCa^{2+}uptake, human MCU can assemble in a functional channel only when EMRE is present [9].

A critical aspect of mitCa^{2+} uptake is the sigmoidal response to cytCa^{2+} levels [10]. At low $[Ca^{2+}]_{cyt}$ concentration, mitCa^{2+}uptake is negligible, despite the huge driving force ensured by the ΔΨm. When $[Ca^{2+}]_{cyt}$ rises, the speed and amplitude of mitCa^{2+} uptake exponentially increase. The proteins responsible for the sigmoidal response to extra- mitochondrial Ca^{2+}belong to the MICU family. MICU1 is a 54 kDa protein and was the first member of the MCUC to be identified [11]. It has an N-terminal mitochondrial targeting sequence and two EF-hand Ca^{2+} binding domains ensuring a dual-mode control on MCU. When extra-mitochondrial [Ca^{2+}] is low, MICU1 keeps the channel close to avoid constant Ca^{2+} entry, as demonstrated by the fact that the silencing of MICU1 causes mitCa^{2+}overload [12,13]. On the other hand, when extra-mitochondrial [Ca^{2+}] exceeds a certain threshold, MICU1 acts as a cooperative activator of MCU, thus ensuring high MCU-mediated Ca^{2+} conductivity upon cell stimulation [8,12,13].

Two MICU1 paralogs located in the IMS contribute to the fine regulation of mitCa^{2+} entry, i.e. MICU2 and MICU3. Moreover, an alternative splice variant of MICU1, i.e. MICU1.1, is specifically expressed in the skeletal muscle and, at lower levels, in the brain [14]. MICU2, formerly named EFHA1, displays a tissue expression pattern similar to MICU1 [15]. Instead, MICU3, formerly named EFHA2, is expressed prevalently in specific tissues, such as the nervous system and the skeletal muscle [15,16]. As MICU1, MICU2 contains two conserved EF-hand domains. MICU2 forms obligate heterodimers with MICU1, stabilized by a disulfide bond through two conserved cysteine residues [8,12]. At low $[Ca^{2+}]_{cyt}$ MICU2 can inhibit MCU activity [8]. Alternatively, it was proposed that both MICU1 and MICU2 exert gatekeeping properties and that, in the absence of MICU2, mitCa^{2+}uptake is still inhibited by MICU1 [17]. Other studies indicate that the main function of MICU2 is to regulate the Ca^{2+} threshold of channel activation [18]. Moreover, in various cell types, MICU2 protein stability, and thus its activity, depends on the presence of MICU1 [8,15]. Their relative role is better clarified by structural studies, as detailed in the next session. Functionally, the crosstalk between MICU1 and MICU2 becomes evident in tissues in which their expression differs significantly. MICU1 is highly expressed in liver and skeletal muscle, while MICU2 expression is abundant in the heart. Thus, in liver and skeletal muscle, on one hand the threshold for mitCa^{2+} entry is elevated to avoid deleterious intra-organellar ions overload, on the other hand high cooperativity for Ca^{2+} entry is reached, which is functional to ensure efficient oxidative metabolism and ATP production. The relatively high amount of MICU2 of cardiac mitochondria is instrumental to reduce mitCa^{2+}uptake upon cytCa^{2+} elevation, allowing the decoding of the repetitive cytCa^{2+} spikes of the beating heart into a graded increase of matrix Ca^{2+} [16,19].

Loss-of-function mutations or deletions of MICU1 have been detected in patients affected by myopathy, weakness, fatigability, and by learning difficulties and lethargy [20–22]. In patient fibroblasts, MICU1 mutations cause increased resting $[Ca^{2+}]_{mit}$, increased rate of mitCa^{2+} uptake upon stimulation but no difference in $[Ca^{2+}]_{mit}$ peak [20]. An in-depth analysis of mitochondrial ion dynamics demonstrated that a futile mitochondria Ca^{2+} cycle occurs in MICU1-depleted patient fibroblasts, whereby the mitochondrial Na^+/Ca^{2+} exchanger (NCLX) is activated, triggering Ca^{2+} efflux. The consequent increase in Na^+ entrance in turn stimulates the Na^+/H^+ exchange, altering the H^+ gradient required for ATP production [23]. It is plausible that the energetic costs of this futile Ca^{2+} cycle undermine oxidative phosphorylation leading to a bioenergetics deficit that has a major impact on neurons and muscles.

Of note, also MICU2 loss-of-function was associated with a severe defect in neurodevelopment [24].

An alternative splicing variant of MICU1, i.e. MICU1.1, is expressed in the skeletal muscle, where it represents the predominant variant [14], and in the brain, although at lower levels. MICU1.1 differs from MICU1 for the addition of a micro-exon inserted between exon 5 and exon 6 that greatly modifies the properties of the uniporter [14]. Indeed, MICU1.1 overexpression triggers a major increase of agonist-induced mitCa^{2+} uptake that exceeds that caused by the overexpression of conventional MICU1. Moreover, in skeletal muscle, the MICU1.1–MICU2 dimer enhances the ability of mitochondria to take up Ca^{2+}, and increases ATP production [14]. This effect is not due to differences in the ability of MICU1.1 to interact with MCU, to heterodimerize with MICU2 or to form high molecular weight complexes.

MICU3 is a 55 kDa protein sharing 34% and 47% protein sequence similarity with MICU1 and MICU2, respectively, and is mainly expressed in the nervous system [16]. Like MICU1 and MICU2, MICU3 has two EF-hand domains and a mitochondrial targeting signal. Nonetheless, MICU3 displays a reduced gatekeeping activity compared to MICU1, and mediates a more rapid response to intracellular Ca^{2+} changes, allowing a shorter delay between the intracellular Ca^{2+}rise and the onset of mit-Ca^{2+}uptake [8]. The fine tuning of Ca^{2+} sensitivity exerted by MICU3 in presynaptic mitochondria warrants ATP synthesis in response to small cytCa^{2+} increases, thus ensuring the metabolic flexibility required for axonal conductance [25]. In addition, non-negligible amounts of MICU3 are present in heart mitochondria. In agreement with the role of MICU3 in facilitating MICU1-mediated channel activation, loss of MICU3 confers cardioprotection against isoproterenol-induced dysfunction and ischemia-reperfusion injury, thus suggesting a MICU3-dependent regulation of pathological mitCa^{2+}overload in the heart [26].

MCUR1 is an IMM protein of about 40 kDa. Both the N- and the C-termini of the protein extend into the IMS, while most of the protein sequence is in the matrix. The transmembrane domain is composed of two helices. Within the matrix, a Ca^{2+}-binding head domain is required for MCUR1-MCU interaction and is connected by a β-layer neck to a coiled-coiled stalk anchored to the membrane [27]. Downregulation of MCUR1 decreases mitCa^{2+} uptake, oxidative phosphorylation, and ATP production [28]. MCUR1 ensures proper assembly of the nascent MCUC, while being possibly dispensable for the mature channel comprising MICU1 [29]. Nonetheless, the negative effects of MCUR1 down-regulation on mitCa^{2+} uptake and oxidative phosphorylation have been questioned and have been alternatively attributed to the role that MCUR1 plays on the assembly of cytochrome-c oxidase [30]. In line with this hypothesis, but differently from previous reports [28,31], the ΔΨm required for mitochondrial Ca^{2+} entry was dissipated in MCUR1 depleted fibroblasts [30]. The demonstration that MCUR1 regulates MCU activity with negligible effect on ΔΨm was provided by patch clamp recordings of MCU-mediated Ca^{2+} currents in MCUR1-silenced mitoplasts. In conditions of tightly controlled ΔΨm, MCUR1 knock-down diminished MCU-mediated Ca^{2+} currents [32]. Moreover, both cardiac and endothelial mitochondria of tissue-specific MCUR1$^{-/-}$ mice showed minimal mitCa^{2+} uptake upon extramitochondrial Ca^{2+} pulses despite constant ΔΨm [33]. MCUR1 levels also modulate the Ca^{2+} threshold for activation of the mitochondrial permeability transition, thus playing a role in cell viability [34]. Notably, MCU-independent functions have been attributed to MCUR1, including proline metabolism in yeast [35], in line with the non-overlapping expression of MCU and MCUR1 in different species.

Finally, Ca^{2+} efflux pathways are needed to limit mitochondrial Ca^{2+} accumulation and avoid the deleterious consequence of Ca^{2+} overload. The Na^+/Ca^{2+} mitochondrial exchanger, NCLX, is responsible for mitochondrial Ca^{2+} efflux [36], and protects from myocardial and neuronal dysfunction in vivo [37,38].

Recently, TMBIM5 was proposed as a novel member of the mitochondrial ion transport system [39–41]whose loss triggers embryonic or perinatal lethality and skeletal myopathy associated to tissue-specific

disruption of cristae architecture and opening of the mitochondrial permeability transition pore [39]. However, the role of TMBIM5 in controlling mitochondrial Ca^{2+} is controversial. It has been proposed that TMBIM5 overexpression increases mitochondrial Ca^{2+} uptake, but its downregulation has little effect on Ca^{2+} homeostasis. Rather, TMBIM5 deficiency attenuates K^+/H^+ exchange activity [39]. Alternatively, TMBIM5 was proposed as a Ca^{2+}/H^+ antiporter [40,41]. In particular, T. Langer's lab proposed TMBIM5 as an interactor of the m-AAA protease AFG3L2 and as a Ca^{2+}/H^+ exchanger allowing Ca^{2+} efflux from mitochondria and limiting mitochondria hyperpolarization [41].

2. ACT II. Enough is enough: proteolytic regulation of EMRE controls complex assembly and activity

As discussed above, the MCU activity must be tightly regulated to prevent mitCa^{2+} overload, a condition that triggers the opening of the mPTP, eventually leading to cell death [10]. This fine regulation relies on the MICU family of EF-hand containing proteins that, on one hand, inhibits MCU opening in resting cytCa^{2+}concentrations to prevent ion futile cycles and, on the other, allows the opening of the channel when a specific threshold of cytCa^{2+} concentration is reached [8]. In this scenario, EMRE plays a crucial role for the correct assembly of MCUC and in preventing mitCa^{2+} overload. Langer's laboratory proposed a model in which the assembly of MCUC is based on a two-step mechanism (Fig. 1). Firstly, EMRE interacts with the regulatory complex formed by MICU1 and MICU2 and, subsequently, this intermediate complex assembles with the pore-forming subunits MCU thus creating a functionally regulated MCUC [42]. This is a critical step since excessive amount of EMRE leads to the formation of a constitutively active EMRE-MCU complex, resulting in mitCa^{2+} overload [42]. EMRE protein levels are controlled by proteolytic processing ensured by different members of the AAA family of enzymes (ATPases associated with diverse cellular activities), ATP-dependent proteases embedded in the IMM [43]. According to their topology, these proteases can be divided in *i*- and *m*-AAA proteases. *i*-AAA proteases expose their catalytic domain to the IMS, while *m*-AAA proteases to the matrix side. In humans, *i*-AAA proteases are formed by homo-oligomeric complexes of YME1 Like 1 ATPase (YME1L) subunits, while *m*-AAA proteases can be formed by homo-oligomeric complexes of AFG3 ATPase family gene 3-like 2 (AFG3L2) or hetero-oligomeric complexes of AFG3L2 and spastic paraplegia 7 (SPG7) [43]. It was recently shown that, during mitochondrial import, newly synthesized EMRE binds to the Matrix AAA Peptidase Interacting Protein 1 (MAIP1), a binding partner of *m*-AAA proteases, that protects precursor EMRE from the degradation by the *i*-AAA protease YME1L in the IMS. Therefore, MAIP1 acts as a chaperon-like protein, although the molecular mechanism has not been fully clarified. After maturation by the general mitochondrial processing peptidase MPP in the mitochondrial matrix, mature EMRE is inserted into the IMM and forms a complex with MICU1/MICU2. In physiological conditions, non-assembled EMRE is rapidly degraded by the *m*-AAA protease AFG3L2, thus preventing the formation of constitutively active EMRE-MCU complexes [42,44]. Several pieces of evidence underline the critical role of AFG3L2 in the regulation of EMRE protein levels and thus mitCa^{2+} uptake. Indeed, protease-resistant EMRE variants were shown to escape the proteolytic degradation by the *m*-AAA protease leading, as expected, to the formation of Ca^{2+}-leaky constitutively active MCUC [45]. Furthermore, mutations in the *m*-AAA protease AFG3L2 gene associated to the neurodegenerative disorder spinocerebellar ataxia (SCA28) cause the loss of the m-AAA proteolytic regulation of EMRE, with consequent mitCa^{2+} overload triggering Purkinje cells death [42]. Collectively, these results provide a model in which the fine proteolytic regulation of EMRE is crucial for mitCa^{2+} homeostasis through the regulation of MCUC assembly. Further studies are needed to understand whether the

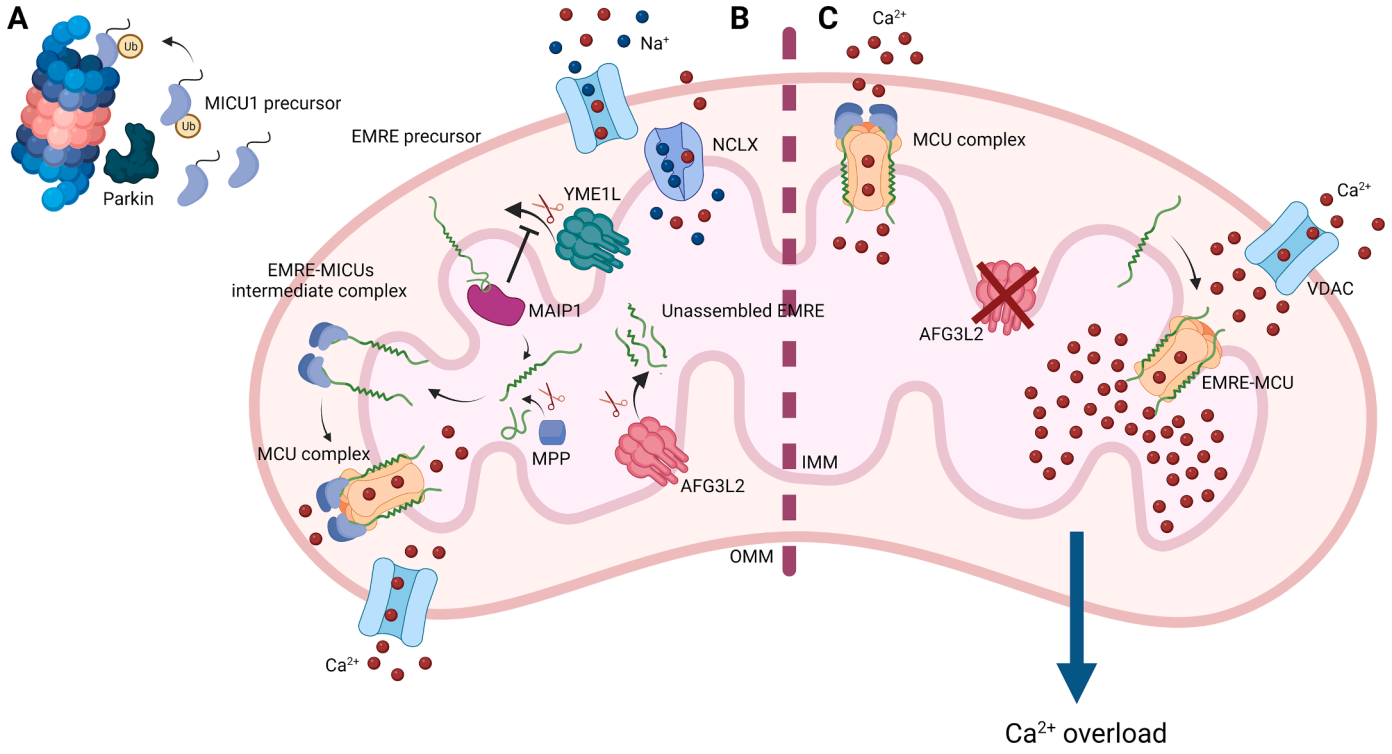


Fig. 1. A) The E3 Ubiquitin ligase Parkin selectively targets cytosolic precursor MICU1 for degradation by the Ubiquitin Proteasome System (UPS). B) The binding of MAIP1 protects precursor EMRE from the degradation by YME1L in the IMS. After MPP cleavage in the mitochondrial matrix, mature EMRE is inserted into the IMM where it forms a complex with MICU1/MICU2. This intermediate complex then assembles with MCU, creating a functionally regulated complex. Non-assembled EMRE in the IMM is degraded by the m-AAA AFG3L2. C) Loss of AFG3L2 proteolytic regulation of EMRE causes the formation of constitutively active EMRE-MCU complexes, leading to mitochondrial matrix Ca^{2+} overload. (VDAC: voltage-gated anion channel; NCLX: mitochondrial Na^+/Ca^{2+} exchanger).

proteolytic regulation of EMRE could impinge in the stoichiometry of MCUC that seems to be fundamental for its channel activity. Indeed, Foskett's laboratory recently showed that the 4:4 MCU:EMRE stoichiometry is not obligatory for mediating Ca^{2+} uptake but MICU1/2 tethering to the complex, and thus MCU activity, might be modulated by the number of EMRE subunits per channel [46].

In spite of the extensive knowledge on the gatekeeping mechanisms of the mitochondrial Ca^{2+} channel, how the import, the folding, the stability and the degradation of MCUC proteins are regulated at the post-translational level is much less clear. In this regard, also MICU1 is a substrate of the *i*-AAA protease YME1L [47], but nothing is known on the mechanism by which MICU2 protein stability depends on that of MICU1, as well as on the regulation of the assembly of the functional channel.

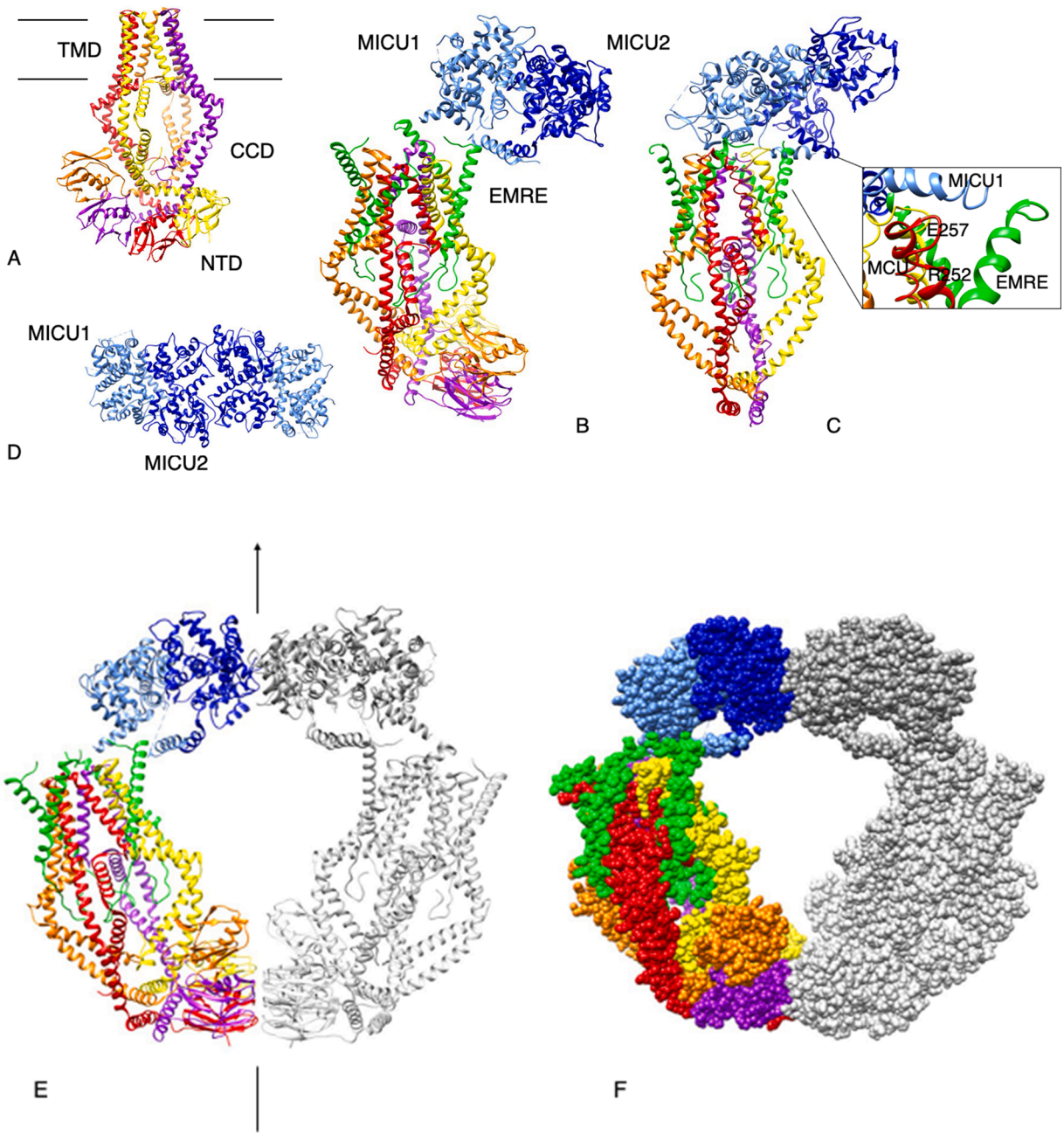


Fig. 2. A) Cartoon model of human MCU tetramer. The four monomers are colored red, yellow, orange and magenta. The three domains, TMD, CCD and NTD, are labelled. The four-fold symmetry axis runs vertical, in the plane of the paper. The coordinates are from model PDB ID 6k7y [58]. B) The monomer of the supercomplex at high-calcium concentration, including MCU (same colors as in A), EMRE (green), MICU1 (light blue), and MICU2 (blue). C) The monomer of the supercomplex at low-calcium concentration (coordinates from the model PDB ID 6wdn, [55]). The inset shows a detail of the interaction of one MCU monomer (red), of EMRE (green), and of MICU1 (light blue), where the positions of residues Arg 252 and Glu 257 of MCU, forming a salt bridge, are indicated (the orientation of the inset is slightly rotated with respect to the whole model). D) The two dimers MICU1-MICU2, coordinates from the high-calcium concentration in the dimeric uniporter supercomplex. MICU1-MICU2 are oriented face-to-face, MICU2 – MICU2 back-to-back. E) Cartoon view of the dimeric uniporter supercomplex in the high-calcium concentration (coordinates from model PDB ID 6k7y [58]). The O-shape of the entire complex is clearly visible, with two monomers related by a two-fold axis represented by the arrows that run from the bottom to the top of the paper. In the monomer on the left the colors are as in B. F) Space filling representation.

3. ACT III. A mine of gemstones: what the 3D-structures reveal about the MCU complex

Several structures of MCU, MICU1, MICU2, and of their complexes from different organisms are present in the Protein Data Bank (PDB, www.rcsb.org). They include the crystal structure of MICU1 Ca^{2+}-free and Ca^{2+}-bound (PDB ID 4nsc, [48]), the *apo* structure of MICU2 dimer (PDB ID 6eaz, [49]), that of Ca^{2+}-bound MICU2 fused to the T4 bacteriophage lysozyme (PDB ID 6iih, [50]), and that of the *apo* MICU1-MICU2 [51]. The structures of MCU from lower species include MCU from *Cyphellophora europaea* and Zebrafish (PDB ID 6dnf [3]), from *Neosartorya fischeri* (PDB ID 6d7w, 6d80, [52]), and from *Metarhizium acridum* (PDB ID 6c5w and 6c5r, [53]). Finally, thanks to the use of cryo-EM, some structures of MCUC are now available at near-atomic resolution: a. the human MCU in complex with EMRE (PDB ID 6o58, [54]), b. the entire human mitochondrial complex including MCU-EMRE-MICU1-MICU2 in the inhibited and in the Ca^{2+}-activated states (PDB ID 6wdn, 6wdo, [55]), c. the complex in the presence and absence of Ca^{2+} (PDB ID 6xjv, [56]), d. the holo-complex of beetle *Tribolium castaneum* (PDB ID 6xqn, 6xqo, [57]), and e. the human complex expressed in HEK 293F cells (PDB ID 6k7y, 6k7x, [58]). These structures altogether reveal the overall architecture of MCUC and the gatekeeping mechanism.

In all organisms analyzed till now, MCU assembles as a tetramer with a common overall architecture in all organisms, despite some minor differences. Each monomer can be described as made by three domains: an N-terminal domain (NTD), a coiled-coil domain (CCD), and a transmembrane domain (TMD) (Fig. 2A). Each TMD, responsible for Ca^{2+} conduction, is made by eight α-helices, two from each subunit. One α-helix from each subunit contributes to form the inner wall of the channel. The conserved WDIMEP sequence represents the MCU Ca^{2+} channel signature. In the high-Ca^{2+} structures of the uniporter supercomplex (6k7y, 6wdo [55,58]), one Ca^{2+} ion is bound at the entrance of the channel, coordinated by the eight oxygen atoms of the four E264 residues. The other two MCU domains, CCD and NTD, are located in the mitochondrial matrix. NTD is a barrel of six β-strands with an α-helix interspersed in between strands β3 and β4. CCD has three long helices, one of which connects NTD to TMD, and the function of this domain seems to be that of stabilizing the conformation of the MCU tetramer [53].

Each monomer forming the MCU tetramer makes an extensive interaction with EMRE, a small, largely hydrophobic polypeptide, consisting of a long α-helix and a N-terminal β-hairpin.

The two regulatory subunits MICU1 and MICU2, located in the intermembrane space, present a very similar core structure, made by two lobes with a high α-helical content (Fig. 2B - D) [48–51]. Each of them contains four EF-hands, of which only two are able to bind Ca^{2+}. MICU1 and MICU2 form a face-to-face dimer that interacts with the lipids of the membrane through their C-terminal helices [54–58].

At high- Ca^{2+} concentration, the uniporter supercomplex appears to be in a dimeric form, whose stoichiometry is 2 x (4:4:1:1) for MCU: EMRE:MICU1:MICU2, corresponding to a molecular mass of about 480 kDa. Two MCU channels form an O-shaped dimer characterized by a two-fold symmetry axis roughly perpendicular to the plane of the membrane (Fig. 2E and F [55]). It is worth to observe that the two transmembrane arms of this O-shaped supercomplex need to be bent, with a positive curvature, to be roughly perpendicular to the membrane [56]. The interactions between the two monomers involve the NTD of MCU in the mitochondrial matrix and two MICU2 monomers in the intermembrane space. In the former, the interaction is staggered and quite extended: NTD1 of monomer 1 interacts with NTD3 of monomer 2, NTD2 of monomer 1 with NTD2 of monomer 2, and NTD3 of monomer 1 with NTD1 of monomer 2. In the latter, the interaction between the two MICU2 subunits is back-to-back, whilst the opposite surface of MICU2 interacts with MICU1 [55–58].

In the high- Ca^{2+} structures of the uniporter, the interaction of MICU1 with MCU is mediated mostly by the negatively charged C-terminal tail of one EMRE subunit bound to the MCU tetramer and by some limited contacts with a second EMRE subunit, without direct interaction MICU1-MCU [58]. It is worth noticing that the indirect interaction of the MCU tetramer with one single MICU1 breaks the tetrameric symmetry of MCU [56].

The structures available of the low- Ca^{2+} models of the uniporter correspond to the monomeric form, i.e. the structure with a stoichiometry 4:4:1:1 of the components (6wdn, [55,56]; Fig. 2C). This suggests that the conformational modification of the MICU1-MICU2 complex triggered by the absence of Ca^{2+} bound [48,49,51] that induces a significant change of the interaction surface of MICU1 with MCU, alters the stability of the dimeric supercomplex, shifting its equilibrium towards the monomeric form. In the low- Ca^{2+} structure, an extensive surface of interaction of MICU1 with MCU is present, whilst the interaction with EMRE is reduced to a minimum. The consequence of this large conformational rearrangement of the uniporter supercomplex is that MICU1 now blocks the gate of the Ca^{2+} channel.

It has been suggested that the dissociation of Ca^{2+} ion(s) from one of the MICU heterodimers could induce the dissociation of the same ion from the other, since the conformation of bound and unbound MICUs are incompatible with the formation of the MICU1-MICU2 dimer [55]. This could justify the cooperative activation of Ca^{2+} on the uniporter.

If this hypothetical gating mechanism is correct, rather than switching on/off the Ca^{2+} conductance by altering the conformation of the channel, at low- Ca^{2+} concentration ($Ca^{2+} < 1$ μM) MICU1 shuts the channel by covering the MCU pore entrance. As Ca^{2+} concentration increases, the MICU1-MICU2 heterodimer modifies its conformation by binding Ca^{2+} cooperatively and, as a consequence, the interaction MICU1-MCU weakens. The heterodimer moves away from the entrance of the pore and binds MCU in a way mediated by EMRE, leading to the full activation of the uniporter [55,56]. It has also been suggested [58] that the conformational change of MICU1/MICU2 following the binding of Ca^{2+} ions modifies the structure of the complex by pushing the N-terminus of EMRE. The latter works as a lever, modifying the conformation of the channel and/or stabilizing the open state.

As introduced in Act I, MCUb, a variant characterized by mutations R252W and E257V, has been proposed as an endogenous negative dominant of MCU [4]. The two mutated residues are located in the TMD, at the end of the first α-helix that forms the Ca^{2+} channel, just before the loop that defines the upper surface of the MCU tetramer. In the high Ca^{2+} structure, the side chains of Arg 252 and Glu 257 of MCU form between them a strong salt bridge, and they also interact with residue Lys 86 of EMRE, forming a cluster of charged residues (Fig. 2C). In addition, in the structure of the uniporter supercomplex (PDB ID 6k7y) the side chain of Phe 106 of MICU1 is at close distance from Glu 257 of MCU. In summary, this area appears crucial for the triple interaction MCU-EMRE-MICU1 and the double MCUb mutation could destabilize the interaction, in particular that of the C-terminus of EMRE with MCU. It must be noticed that, since the interaction with MICU1 involves only two MCU monomers in the tetramer, in case of the presence of both MCU and MCUb in one tetramer, Ca^{2+} transport could be mitigated and not necessarily fully abolished.

4. ACT IV. Refining the roles: post-translational modifications contribute to the fine-tuning of MCU activity

MCU protein undergoes a variety of post-translational modifications including phosphorylation, S-glutathionylation, and methylation.

In cardiac myoblasts upon adrenoceptor stimulation, tyrosine phosphorylation of MCU by proline-rich tyrosine kinase 2 (Pyk2) was reported [59]. Pyk2-dependent phosphorylation promotes MCU channel oligomerization and formation of tetrameric channels, thus enhancing mitCa^{2+} uptake. Therefore, ROS generation is increased and Ca^{2+} overload occurs, initiating the apoptotic signaling cascade [59]. Similar activation of the Pyk2/MCU pathway accompanied by mitochondria

damage and apoptosis was recently detected in the ischemic rat brain after middle cerebral artery occlusion, indicating that the Pyk2/MCU pathway may represent a common mediator of stress-induced mitochondrial damage and cell death [60]. MCU contains 15 tyrosine residues (5 in the N-terminus, 4 in transmembrane domains and 6 in the C-terminus), which are conserved across all eukaryotic species. Phosphorylation prediction programs revealed that only three of these tyrosine residues (Tyr 158 at N-terminus, Tyr 289, and Tyr 317 at C-terminus in mouse MCU) are putative phosphorylation sites for protein tyrosine kinases (PTKs), but only one of these (Tyr 289) is predicted to be a potential Pyk2-specific phosphorylation site [59]. Future studies are needed to definitively determine Pyk2-specific phosphorylation site (s) in MCU [59]. Based on the 3D structure, the three proposed tyrosine residues are all buried, making their phosphorylation unlikely unless a significant conformational modification of the structure takes place.

Joiner et al. [61] proposed that MCU activity increases upon phosphorylation by CAMKII eventually impacting on programmed cell death, and that inhibition of CaMKII activity protects cardiomyocytes from I/R injury [61]. In addition, electrophysiological studies on mitoplasts revealed reduced MCU currents in heart mitochondria of transgenic mice expressing a CAMKII inhibitory peptide compared to wild type mice [61]. Furthermore, absence of ATP or addition of a catalytically incompetent CaMKII mutant (K43M) failed to increase MCU activity. Structural analysis of MCU revealed that Ser 92, a conserved residue in the L2 loop of NTD, is a putative phosphorylation site for CaMKII [62]. According to the 3D model, Ser 92 is positioned on the surface of the NTD, where the dimerization of the two MCU takes place. In contrast with the putative increase in MCU activity, its phosphorylation would possibly prevent the formation of the dimeric O-complex. However, the role of CaMKII in regulating MCU currents has been questioned [63,64], both by patch clamp recordings in mouse heart mitochondria [64], and by assessing mitochondrial Ca^{2+} uptake in CAMKII-deficient animal models [63].

Phosphorylation of MCU by AMPK is necessary for the restoration of ATP levels during cell division [65]. Cell division is a highly energy-demanding cellular process, where the coordination of energy sensing and production is poorly understood. The AMPK-dependent MCU phosphorylation at Ser 57 occurs within mitochondria and activates mitCa^{2+} entry leading to the intensification of mitochondrial respiration and energy production necessary for proper spindle dynamics. AMPK deletion reduced the level of MCU phosphorylation at Ser 57 specifically in mitotic cells, indicating that basal MCU phosphorylation is mediated by other kinases [65]. According to the 3D model of the MCU structure, Ser 57 is located in a flexible area. Its phosphorylation would have no effect on the supercomplex structure, but it could impinge on the interaction of MCU with other partners controlling channel function.

MCU phosphorylation was also proposed to contribute to the development of colorectal cancer (CRC). In particular, receptor-interacting protein kinase 1 (RIPK1), which controls cell fate and is significantly upregulated in CRC cells, interacts with MCU to increase mitCa^{2+}uptake and energy metabolism, eventually promoting CRC cell proliferation [66]. Although the specific phosphorylation residue on MCU is not known, Lys 377 of RIPK2 has been identified as a crucial site for the interaction with MCU.

Within the post transcriptional modifications of MCU, MCU oxidation has been reported [67]. MCU has three conserved cysteines (Cys 67, Cys 97 and Cys 191) in the NTD. Mutagenesis assays identified Cys 97 as the only redox-sensitive residue of the MCU. Accordingly, based on the 3D model, while Cys 67 and Cys 191 cannot be involved in disulfide bridges, and in fact they are in a reduced state, Cys 97 is relatively exposed. Experimental data indicate that, upon oxidation, Cys 97 is reversibly S-glutathionylated, and this would promote, with a mechanism still to be clarified, the assembly of MCU channels into higher-order complexes that exhibit a persistent activity, leading to increased mitCa^{2+} accumulation [67]. Sustained mitCa^{2+} uptake, in turn, increases the production of ROS by mitochondria, leading to cell death. Interestingly, none of the other subunits of the channel (MCUb, MCUR1, MICU1/2, and EMRE) displayed any detectable modification in gel shift assays when exposed to oxidizing agents [67].

A recently described Akt kinase-mediated phosphorylation of MICU1 at the N-terminal region is the first demonstration of a phosphorylation event for the MICU1 subunit [68]. Specifically, the mitochondrial pool of active Akt is responsible for the MICU1 phosphorylation at Ser 124 (Ser 122 in the PDB model), which affects the MICU1 processing leading to MICU1-MICU2 dimer instability. The 3D model of MICU1 structure in the uniporter supercomplex [55] highlights the presence of a long and flexible loop (445–453) facing Ser 122. The presence of a negative charge due to phosphorylation of this site may cause a perturbation of the loop resulting in the destabilization of the MICU1-MICU2 dimer. The loss of MICU1 inhibitory activity on MCU causes a significant increase in the basal mitochondrial matrix Ca^{2+} level, culminating in aberrant ROS production and tumor progression [68]. Phosphorylated MICU1 accumulates in a non-mature form that is rapidly degraded, leading to concomitant loss of the binding partner MICU2 and a disordered MCU complex composition [68]. The expression of a non-phosphorylable MICU1 mutant restores normal mitCa^{2+} and ROS levels and inhibits Akt-mediated tumor growth in vivo, suggesting that the MICU1 dependent regulation of mitCa^{2+} and ROS homeostasis are crucial elements in Akt-driven tumorigenesis.

In addition to phosphorylation, the MICU1 gatekeeper function is also modulated post-translationally through its methylation by arginine methyl transferase 1 (PRMT1) at Arg 455 [69], a residue close to the end of the flexible area in MICU1, thus with limited effects on the MCU supercomplex structure but with putative outcomes on protein-protein interaction events.

5. ACT V. Exploring the complex: small molecules and drugs modulate MCU activity

The most broadly used MCU inhibitor is RU360 [70,71] and its recent derivative Ru265 [72]. The thiourea KB-R7943, initially developed as a negative modulator of the plasma membrane Na^{+}/Ca^{2+} exchanger, also inhibits MCU [73]. By screening small-molecule libraries, other cell-permeable inhibitors of mitCa^{2+} uptake were identified, including DS16570511 [74], mitoxantrone [75] and MCU-i11 [76]. Finally, the FDA approved drug benzethonium was also shown to inhibit MCU [77]. Regarding positive MCU modulators, spermine and related polyamines lower the threshold of mitCa^{2+} uptake [78]. The p38 mitogen-activated protein kinase inhibitor SB202190 modulates mitCa^{2+} with a mechanism independent of p38 activity [79]. In addition, several natural plant flavonoids increase MCU activity independently of their antioxidant activity [80]. Agonists and antagonists of the estrogen receptor as 4,4′,4″-(4-propyl-[1 *h*]-pyrazole-1,3,5-triyl)trisphenol (PPT), diethylstilbestrol, and 17-β-estradiol modulate the activity of the uniporter [81]. Finally, the anti-fungal drug amorolfine was shown to increase mitCa^{2+} both in vitro and in vivo [77].

6. Epilogue

Since the first report of MICU1 as an essential component of the mitCa^{2+} uptake machinery, studies of the last decade have revealed the existence of many different players involved in the formation and in the regulation of the channel. Mutation studies have further uncovered the function of the MCU complex proteins and of their post-translational modifications, further corroborated by the disease phenotype of patients carrying MICU1 or MICU2 mutations. In addition, proteolytic events regulate the MCU-EMRE abundance with immediate consequences on channel activity. Crystals and cryo-EM structures help elucidating the complexity of the channel assembly and the relative movement of regulatory elements and specific peptide sequences in the presence or absence of Ca^{2+}. Still, for a full comprehension of the MCU

function, activity and control, further experimental studies and structural data are needed, in particular about partners transiently interacting with the MCU supercomplex.

CRediT authorship contribution statement

Agnese De Mario: Writing – original draft, Writing – review & editing. **Donato D'Angelo:** Writing – original draft, Writing – review & editing. **Giuseppe Zanotti:** Conceptualization, Writing – original draft, Writing – review & editing. **Anna Raffaello:** Conceptualization, Writing – original draft, Writing – review & editing, Funding acquisition. **Cristina Mammucari:** Conceptualization, Writing – original draft, Writing – review & editing, Funding acquisition, Supervision.

Declaration of competing interest

The authors of the manuscript entitled "The Mitochondrial Calcium Uniporter (MCU) complex - A play in five acts" declare no conflict of interest.

Data availability

No data was used for the research described in the article.

Acknowledgements

This research was supported with funding from the Italian Ministry of Health (GR-2016-02362779 to A.R.), the Italian Ministry of University and Research (PRIN 20207P85MH to A.R.), the French Muscular Dystrophy Association (22493 C.M.), the Veneto Region (POR FESR to C.M.). A.D.M. is recipient of a post-doctoral fellowship from the French Muscular Dystrophy Association (22962).

References

[1] J.M. Baughman, F. Perocchi, H.S. Girgis, M. Plovanich, C.A. Belcher-Timme, Y. Sancak, X.R. Bao, L. Strittmatter, O. Goldberger, R.L. Bogorad, V. Koteliansky, V. K. Mootha, Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter, Nature 476 (2011) 341–345, https://doi.org/10.1038/nature10234.

[2] D. De Stefani, A. Raffaello, E. Teardo, I. Szabó, R. Rizzuto, A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter, Nature 476 (2011) 336–340, https://doi.org/10.1038/nature10230.

[3] R. Baradaran, C. Wang, A.F. Siliciano, S.B. Long, Cryo-EM structures of fungal and metazoan mitochondrial calcium uniporters, Nature 559 (2018) 580–584, https://doi.org/10.1038/s41586-018-0331-8.

[4] A. Raffaello, D. De Stefani, D. Sabbadin, E. Teardo, G. Merli, A. Picard, V. Checchetto, S. Moro, I. Szabò, R. Rizzuto, The mitochondrial calcium uniporter is a multimer that can include a dominant-negative pore-forming subunit, EMBO J. 32 (2013) 2362–2376, https://doi.org/10.1038/emboj.2013.157.

[5] F. Fieni, S.B. Lee, Y.N. Jan, Y. Kirichok, Activity of the mitochondrial calcium uniporter varies greatly between tissues, Nat. Commun. 3 (2012) 1317, https://doi.org/10.1038/ncomms2325.

[6] S. Feno, F. Munari, D. Vecellio Reane, R. Gissi, D.-H. Hoang, A. Castegna, B. Chazaud, A. Viola, R. Rizzuto, A. Raffaello, The dominant-negative mitochondrial calcium uniporter subunit MCUb drives macrophage polarization during skeletal muscle regeneration, Sci. Signal. 14 (707) (2021) eabf3838, https://doi.org/10.1126/scisignal.abf3838.

[7] Y. Sancak, A.L. Markhard, T. Kitami, E. Kovács-Bogdán, K.J. Kamer, N.D. Udeshi, S. A. Carr, D. Chaudhuri, D.E. Clapham, A.A. Li, S.E. Calvo, O. Goldberger, V. K. Mootha, EMRE is an essential component of the mitochondrial calcium uniporter complex, Science 342 (2013) (1979) 1379–1382, https://doi.org/10.1126/science.1242993.

[8] M. Patron, V. Checchetto, A. Raffaello, E. Teardo, D. VecellioReane, M. Mantoan, V. Granatiero, I. Szabò, D. DeStefani, R. Rizzuto, MICU1 and MICU2 finely tune the mitochondrial Ca2+ uniporter by exerting opposite effects on MCU activity, Mol. Cell 53 (2014) 726–737, https://doi.org/10.1016/j.molcel.2014.01.013.

[9] E. Kovaćs-Bogdán, Y. Sancak, K.J. Kamer, M. Plovanich, A. Jambhekar, R.J. Huber, M.A. Myre, M.D. Blower, V.K. Mootha, Reconstitution of the mitochondrial calcium uniporter in yeast, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 8985–8990, https://doi.org/10.1073/pnas.1400514111.

[10] R. Rizzuto, D. De Stefani, A. Raffaello, C. Mammucari, Mitochondria as sensors and regulators of calcium signalling, Nat. Rev. Mol. Cell Biol. 13 (2012) 566–578, https://doi.org/10.1038/nrm3412.

[11] F. Perocchi, V.M. Gohil, H.S. Girgis, X.R. Bao, J.E. McCombs, A.E. Palmer, V. K. Mootha, MICU1 encodes a mitochondrial EF hand protein required for Ca2+ uptake, Nature 467 (2010) 291–296, https://doi.org/10.1038/nature09358.

[12] G. Csordás, T. Golenár, E.L. Seifert, K.J. Kamer, Y. Sancak, F. Perocchi, C. Moffat, D. Weaver, S. De la Fuente, R. Bogorad, V. Koteliansky, J. Adijanto, V.K. Mootha, G. Hajnóczky, MICU1 controls both the threshold and cooperative activation of the mitochondrial Ca2+ uniporter, Cell Metab. 17 (2013) 976–987, https://doi.org/10.1016/j.cmet.2013.04.020.

[13] K. Mallilankaraman, P. Doonan, C. Cárdenas, H.C. Chandramoorthy, M. Müller, R. Miller, N.E. Hoffman, R.K. Gandhirajan, J. Molgó, M.J. Birnbaum, B.S. Rothberg, D.O.D. Mak, J.K. Foskett, M. Madesh, MICU1 is an essential gatekeeper for mcu-mediated mitochondrial Ca2+ uptake that regulates cell survival, Cell 151 (2012) 630–644, https://doi.org/10.1016/j.cell.2012.10.011.

[14] D. Vecellio Reane, F. Vallese, V. Checchetto, L. Acquasaliente, G. Butera, V. De Filippis, I. Szabò, G. Zanotti, R. Rizzuto, A. Raffaello, A MICU1 splice variant confers high sensitivity to the mitochondrial Ca2+ uptake machinery of skeletal muscle, Mol. Cell 64 (2016) 760–773, https://doi.org/10.1016/j.molcel.2016.10.001.

[15] M. Plovanich, R.L. Bogorad, Y. Sancak, K.J. Kamer, L. Strittmatter, A.A. Li, H. S. Girgis, S. Kuchimanchi, J. De Groot, L. Speciner, N. Taneja, J. OShea, V. Koteliansky, V.K. Mootha, MICU2, a Paralog of MICU1, resides within the mitochondrial uniporter complex to regulate calcium handling, PLoS ONE 8 (2013), https://doi.org/10.1371/journal.pone.0055785.

[16] M. Paillard, G. Csordás, G. Szanda, T. Golenár, V. Debattisti, A. Bartok, N. Wang, C. Moffat, E.L. Seifert, A. Spät, G. Hajnóczky, Tissue-specific mitochondrial decoding of cytoplasmic Ca2+ signals is controlled by the stoichiometry of MICU1/2 and MCU, Cell Rep. 18 (2017) 2291–2300, https://doi.org/10.1016/j.celrep.2017.02.032.

[17] K.J. Kamer, Z. Grabarek, V.K. Mootha, High-affinity cooperative Ca2+ binding by MICU 1– MICU 2 serves as an on–off switch for the uniporter, EMBO Rep. 18 (2017) 1397–1411, https://doi.org/10.15252/embr.201643748.

[18] R. Payne, H. Hoff, A. Roskowski, J.K. Foskett, MICU2 restricts spatial crosstalk between InsP3R and MCU channels by regulating threshold and gain of MICU1-mediated inhibition and activation of MCU, Cell Rep. 21 (2017) 3141–3154, https://doi.org/10.1016/j.celrep.2017.11.064.

[19] I. Drago, D. De Stefani, R. Rizzuto, T. Pozzan, Mitochondrial Ca2+ uptake contributes to buffering cytoplasmic Ca2+ peaks in cardiomyocytes, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 12986–12991, https://doi.org/10.1073/pnas.1210718109.

[20] C.V. Logan, G. Szabadkai, J.A. Sharpe, D.A. Parry, S. Torelli, A.-M. Childs, M. Kriek, R. Phadke, C.A. Johnson, N.Y. Roberts, D.T. Bonthron, K.A. Pysden, T. Whyte, I. Munteanu, A.R. Foley, G. Wheway, K. Szymanska, S. Natarajan, Z. A. Abdelhamed, J.E. Morgan, H. Roper, G.W.E. Santen, E.H. Niks, W.L. van der Pol, D. Lindhout, A. Raffaello, D. De Stefani, J.T. den Dunnen, Y. Sun, I. Ginjaar, C. A. Sewry, M. Hurles, R. Rizzuto, M.R. Duchen, F. Muntoni, E. Sheridan, Loss-of-function mutations in MICU1 cause a brain and muscle disorder linked to primary alterations in mitochondrial calcium signaling, Nat. Genet. 46 (2014) 188–193, https://doi.org/10.1038/ng.2851.

[21] D. Lewis-Smith, K.J. Kamer, H. Griffin, A.M. Childs, K. Pysden, D. Titov, J. Duff, A. Pyle, R.W. Taylor, P. Yu-Wai-Man, V. Ramesh, R. Horvath, V.K. Mootha, P. F. Chinnery, Homozygous deletion in MICU1 presenting with fatigue and lethargy in childhood, Neurol. Genet. 2 (2016) 1–6, https://doi.org/10.1212/NXG.0000000000000059.

[22] S. Musa, W. Eyaid, K. Kamer, R. Ali, M. Al-Mureikhi, N. Shahbeck, F. Al Mesaifri, N. Makhseed, Z. Mohamed, W.A. Alshehhi, V.K. Mootha, J. Juusola, T. Ben-Omran, A middle eastern founder mutation expands the genotypic and phenotypic spectrum of mitochondrial MICU1 deficiency: a report of 13 patients, JIMD Rep. (2019) 79–83, https://doi.org/10.1007/8904_2018_107.

[23] G. Bhosale, J.A. Sharpe, A. Koh, A. Kouli, G. Szabadkai, M.R. Duchen, Pathological consequences of MICU1 mutations on mitochondrial calcium signalling and bioenergetics, Biochim. Biophys. Acta Mol. Cell. Res. 1864 (2017) 1009–1017, https://doi.org/10.1016/j.bbamcr.2017.01.015.

[24] H.E. Shamseldin, A. Alasmari, M.A. Salih, M.M. Samman, S.A. Mian, T. Alshidi, N. Ibrahim, M. Hashem, E. Faqeih, F. Al-Mohanna, F.S. Alkuraya, A null mutation in MICU2 causes abnormal mitochondrial calcium homeostasis and a severe neurodevelopmental disorder, Brain 140 (2017) 2806–2813, https://doi.org/10.1093/brain/awx237.

[25] G. Ashrafi, J. de Juan-Sanz, R.J. Farrell, T.A. Ryan, Molecular tuning of the axonal mitochondrial Ca2+ uniporter ensures metabolic flexibility of neurotransmission, Neuron 105 (2020) 678–687, https://doi.org/10.1016/j.neuron.2019.11.020, e5.

[26] B.N. Puente, J. Sun, R.J. Parks, M.M. Fergusson, C. Liu, D.A. Springer, A.M. Aponte, J.C. Liu, E. Murphy, MICU3 plays an important role in cardiovascular function, Circ. Res. 127 (2020) 1571–1573, https://doi.org/10.1161/CIRCRESAHA.120.317177.

[27] J. Adlakha, I. Karamichali, J. Sangwallek, S. Deiss, K. Bär, M. Coles, M. D. Hartmann, A.N. Lupas, B. Hernandez Alvarez, Characterization of MCU-Binding Proteins MCUR1 and CCDC90B — Representatives of a Protein Family Conserved in Prokaryotes and Eukaryotic Organelles, Structure 27 (2019) 464–475, https://doi.org/10.1016/j.str.2018.11.004, e6.

[28] K. Mallilankaraman, C. Cardenas, P.J. Doonan, H.C. Chandramoorthy, K.M. Irrinki, T. Golenar, G. Csordas, P. Madireddi, J. Yang, M. Müller, R. Miller, J.E. Kolesar, J. Molgo, B. Kaufman, G. Hajnoczky, J.K. Foskett, M. Madesh, Erratum: MCUR1 is an essential component of mitochondrial Ca2+ uptake that regulates cellular metabolism, Nat. Cell Biol. 17 (2015) 953, https://doi.org/10.1038/ncb3202.

[29] J.F. Garbincius, J.W. Elrod, Mitochondrial calcium exchange in physiology and disease, Physiol. Rev. 102 (2022) 893–992, https://doi.org/10.1152/physrev.00041.2020.
[30] V. Paupe, J. Prudent, E.P. Dassa, O.Z. Rendon, E.A. Shoubridge, CCDC90A (MCUR1) is a cytochrome c oxidase assembly factor and not a regulator of the mitochondrial calcium uniporter, Cell Metab. 21 (2015) 109–116, https://doi.org/10.1016/j.cmet.2014.12.004.
[31] H. Rottenberg, A. Scarpa, Calcium uptake and membrane potential in mitochondria, Biochemistry 13 (1974) 4811–4817, https://doi.org/10.1021/bi00720a020.
[32] H. Vais, J.E. Tanis, M. Müller, R. Payne, K. Mallilankaraman, J.K. Foskett, MCUR1, CCDC90A, is a regulator of the mitochondrial calcium uniporter, Cell Metab. 22 (2015) 533–535, https://doi.org/10.1016/j.cmet.2015.09.015.
[33] D. Tomar, Z. Dong, S. Shanmughapriya, D.A. Koch, T. Thomas, N.E. Hoffman, S. A. Timbalia, S.J. Goldman, S.L. Breves, D.P. Corbally, N. Nemani, J.P. Fairweather, A.R. Cutri, X. Zhang, J. Song, F. Jaña, J. Huang, C. Barrero, J.E. Rabinowitz, T. S. Luongo, S.M. Schumacher, M.E. Rockman, A. Dietrich, S. Merali, J. Caplan, P. Stathopulos, R.S. Ahima, J.Y. Cheung, S.R. Houser, W.J. Koch, V. Patel, V. M. Gohil, J.W. Elrod, S. Rajan, M. Madesh, MCUR1 is a scaffold factor for the MCU complex function and promotes mitochondrial bioenergetics, Cell Rep. 15 (2016) 1673–1685, https://doi.org/10.1016/j.celrep.2016.04.050.
[34] D. Chaudhuri, D.J. Artiga, S.A. Abiria, D.E. Clapham, Mitochondrial calcium uniporter regulator 1 (MCUR1) regulates the calcium threshold for the mitochondrial permeability transition, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) E1872–E1880, https://doi.org/10.1073/pnas.1602264113.
[35] M. Zulkifli, J.K. Neff, S.A. Timbalia, N.M. Garza, Y. Chen, J.D. Watrous, M. Murgia, P.P. Trivedi, S.K. Anderson, D. Tomar, R. Nilsson, M. Madesh, M. Jain, V.M. Gohil, Yeast homologs of human MCUR1 regulate mitochondrial proline metabolism, Nat. Commun. 11 (2020) 4866, https://doi.org/10.1038/s41467-020-18704-1.
[36] R. Palty, W.F. Silverman, M. Hershfinkel, T. Caporale, S.L. Sensi, J. Parnis, C. Nolte, D. Fishman, V. Shoshan-Barmatz, S. Herrmann, D. Khananshvili, I. Sekler, NCLX is an essential component of mitochondrial Na+/Ca2+ exchange, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 436–441, https://doi.org/10.1073/pnas.0908099107.
[37] T.S. Luongo, J.P. Lambert, P. Gross, M. Nwokedi, A.A. Lombardi, S. Shanmughapriya, A.C. Carpenter, D. Kolmetzky, E. Gao, J.H. van Berlo, E.J. Tsai, J.D. Molkentin, X. Chen, M. Madesh, S.R. Houser, J.W. Elrod, The mitochondrial Na+/Ca2+ exchanger is essential for Ca2+ homeostasis and viability, Nature 545 (2017) 93–97, https://doi.org/10.1038/nature22082.
[38] P. Jadiya, D.W. Kolmetzky, D. Tomar, A. Di Meco, A.A. Lombardi, J.P. Lambert, T. S. Luongo, M.H. Ludtmann, D. Praticò, J.W. Elrod, Impaired mitochondrial calcium efflux contributes to disease progression in models of Alzheimer's disease, Nat. Commun. 10 (2019), https://doi.org/10.1038/s41467-019-11813-6.
[39] L. Zhang, F. Dietsche, B. Seitaj, L. Rojas-Charry, N. Latchman, D. Tomar, R.C. I. Wüst, A. Nickel, K.B.M. Frauenknecht, B. Schoser, S. Schumann, M.J. Schmeisser, J. vom Berg, T. Buch, S. Finger, P. Wenzel, C. Maack, J.W. Elrod, J.B. Parys, G. Bultynck, A. Methner, TMBIM5 loss of function alters mitochondrial matrix ion homeostasis and causes a skeletal myopathy, Life Sci. Alliance 5 (10) (2022), e202201478, https://doi.org/10.26508/lsa.202201478.
[40] S. Austin, R. Mekis, S.E.M. Mohammed, M. Scalise, W. Wang, M. Galluccio, C. Pfeiffer, T. Borovec, K. Parapatics, D. Vitko, N. Dinhopl, N. Demaurex, K. L. Bennett, C. Indiveri, K. Nowikovsky, TMBIM5 is the Ca2+ /H+ antiporter of mammalian mitochondria, EMBO Rep. 23 (12) (2022) e54978, https://doi.org/10.15252/embr.202254978.
[41] M. Patron, D. Tarasenko, H. Nolte, L. Kroczek, M. Ghosh, Y. Ohba, Y. Lasarzewski, Z.A. Ahmadi, A. Cabrera-Orefice, A. Eyiama, T. Kellermann, E.I. Rugarli, U. Brandt, M. Meinecke, T. Langer, Regulation of mitochondrial proteostasis by the proton gradient, EMBO J. 41 (16) (2022), e110476, https://doi.org/10.15252/embj.2021110476.
[42] T. König, S.E. Tröder, K. Bakka, A. Korwitz, R. Richter-Dennerlein, P.A. Lampe, M. Patron, M. Mühlmeister, S. Guerrero-Castillo, U. Brandt, T. Decker, I. Lauria, A. Paggio, R. Rizzuto, E.I. Rugarli, D. De Stefani, T. Langer, The m-AAA protease associated with neurodegeneration limits MCU activity in mitochondria, Mol. Cell 64 (2016) 148–162, https://doi.org/10.1016/j.molcel.2016.08.020.
[43] F. Gerdes, T. Tatsuta, T. Langer, Mitochondrial AAA proteases - Towards a molecular understanding of membrane-bound proteolytic machines, Biochim. Biophys. Acta Mol. Cell Res. 1823 (2012) 49–55, https://doi.org/10.1016/j.bbamcr.2011.09.015.
[44] M. Patron, H.G. Sprenger, T. Langer, M-AAA proteases, mitochondrial calcium homeostasis and neurodegeneration, Cell Res. 28 (2018) 296–306, https://doi.org/10.1038/cr.2018.17.
[45] C.W. Tsai, Y. Wu, P.C. Pao, C.B. Phillips, C. Williams, C. Miller, M. Ranaghan, M. F. Tsai, Proteolytic control of the mitochondrial calcium uniporter complex, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 4388–4393, https://doi.org/10.1073/pnas.1702938114.
[46] R. Payne, C. Li, J.K. Foskett, Variable assembly of EMRE and MCU creates functional channels with distinct gatekeeping profiles, IScience 23 (4) (2020), 101037, https://doi.org/10.1016/j.isci.2020.101037.
[47] T. MacVicar, Y. Ohba, H. Nolte, F.C. Mayer, T. Tatsuta, H.G. Sprenger, B. Lindner, Y. Zhao, J. Li, C. Bruns, M. Krüger, M. Habich, J. Riemer, R. Schwarzer, M. Pasparakis, S. Henschke, J.C. Brüning, N. Zamboni, T. Langer, Lipid signalling drives proteolytic rewiring of mitochondria by YME1L, Nature 575 (2019) 361–365, https://doi.org/10.1038/s41586-019-1738-6.
[48] L. Wang, X. Yang, S. Li, Z. Wang, Y. Liu, J. Feng, Y. Zhu, Y. Shen, Structural and mechanistic insights into MICU1 regulation of mitochondrial calcium uptake, EMBO J. 33 (2014) 594–604, https://doi.org/10.1002/embj.201386523.
[49] K.J. Kamer, W. Jiang, V.K. Kaushik, V.K. Mootha, Z. Grabarek, Crystal structure of MICU2 and comparison with MICU1 reveal insights into the uniporter gating mechanism, Proc. Natl. Acad. Sci. U S A. 116 (2019) 3546–3555, https://doi.org/10.1073/pnas.1817759116.
[50] W. Wu, Q. Shen, Z. Lei, Z. Qiu, D. Li, H. Pei, J. Zheng, Z. Jia, The crystal structure of MICU2 provides insight into Ca2+ binding and MICU1- MICU2 heterodimer formation, EMBO Rep. 20 (2019) 47488, https://doi.org/10.15252/embr.201847488.
[51] J. Park, Y. Lee, T. Park, J.Y. Kang, S.A. Mun, M. Jin, J. Yang, S.H. Eom, Structure of the MICU1-MICU2 heterodimer provides insights into the gatekeeping threshold shift, IUCrJ 7 (2020) 355–365, https://doi.org/10.1107/S2052252520001840.
[52] N.X. Nguyen, J.P. Armache, C. Lee, Y. Yang, W. Zeng, V.K. Mootha, Y. Cheng, X. chen Bai, Y. Jiang, Cryo-EM structure of a fungal mitochondrial calcium uniporter, Nature 559 (2018) 570–574, https://doi.org/10.1038/s41586-018-0333-6.
[53] C. Fan, M. Fan, B.J. Orlando, N.M. Fastman, J. Zhang, Y. Xu, M.G. Chambers, X. Xu, K. Perry, M. Liao, L. Feng, X-ray and cryo-EM structures of the mitochondrial calcium uniporter, Nature 559 (2018) 575–579, https://doi.org/10.1038/s41586-018-0330-9.
[54] Y. Wang, N.X. Nguyen, J. She, W. Zeng, Y. Yang, X. chen Bai, Y. Jiang, Structural Mechanism of EMRE-Dependent Gating of the Human Mitochondrial Calcium Uniporter, Cell 177 (2019) 1252–1261, https://doi.org/10.1016/j.cell.2019.03.050, e13.
[55] M. Fan, J. Zhang, C.W. Tsai, B.J. Orlando, M. Rodriguez, Y. Xu, M. Liao, M.F. Tsai, L. Feng, Structure and mechanism of the mitochondrial Ca2+ uniporter holocomplex, Nature 582 (2020) 129–133, https://doi.org/10.1038/s41586-020-2309-6.
[56] Y. Wang, Y. Han, J. She, N.X. Nguyen, V.K. Mootha, X.C. Bai, Y. Jiang, Structural insights into the Ca2+-dependent gating of the human mitochondrial calcium uniporter, Elife 9 (2020) 1–19, https://doi.org/10.7554/ELIFE.60513.
[57] C. Wang, A. Jacewicz, B.D. Delgado, R. Baradaran, S.B. Long, Structures reveal gatekeeping of the mitochondrial Ca2+ uniporter by MICU1-MICU2, Elife 9 (2020) 1–30, https://doi.org/10.7554/eLife.59991.
[58] W. Zhuo, H. Zhou, R. Guo, J. Yi, L. Zhang, L. Yu, Y. Sui, W. Zeng, P. Wang, M. Yang, Structure of intact human MCU supercomplex with the auxiliary MICU subunits, Protein Cell 12 (2021) 220–229, https://doi.org/10.1007/s13238-020-00776-w.
[59] J. O-Uchi, B.S. Jhun, S. Xu, S. Hurst, A. Raffaello, X. Liu, B. Yi, H. Zhang, P. Gross, J. Mishra, A. Ainbinder, S. Kettlewell, G.L. Smith, R.T. Dirksen, W. Wang, R. Rizzuto, S.S. Sheu, Adrenergic signaling regulates mitochondrial Ca2+ uptake through Pyk2-dependent tyrosine phosphorylation of the mitochondrial Ca2+ uniporter, Antioxid. Redox. Signal. 21 (2014) 863–879, https://doi.org/10.1089/ars.2013.5394.
[60] K. Zhang, J. Yan, L. Wang, X. Tian, T. Zhang, L. Guo, B. Li, W. Wang, X. Liu, The Pyk2/MCU pathway in the rat middle cerebral artery occlusion model of ischemic stroke, Neurosci. Res. 131 (2018) 52–62, https://doi.org/10.1016/j.neures.2017.09.002.
[61] M.L.A. Joiner, O.M. Koval, J. Li, B. Julie He, C. Allamargot, Z. Gao, E.D. Luczak, D. D. Hall, B.D. Fink, B. Chen, J. Yang, S.A. Moore, T.D. Scholz, S. Strack, P.J. Mohler, W.I. Sivitz, L.S. Song, M.E. Anderson, CaMKII determines mitochondrial stress responses in heart, Nature 491 (2012) 269–273, https://doi.org/10.1038/nature11444.
[62] Y. Lee, C.K. Min, T.G. Kim, H.K. Song, Y. Lim, D. Kim, K. Shin, M. Kang, J.Y. Kang, H. Youn, J. Lee, J.Y. An, K.R. Park, J.J. Lim, J.H. Kim, J.H. Kim, Z.Y. Park, Y. Kim, J. Wang, D.H. Kim, S.H. Eom, Structure and function of the N-terminal domain of the human mitochondrial calcium uniporter, EMBO Rep. 16 (2015) 1318–1333, https://doi.org/10.15252/embr.201540436.
[63] A.G. Nickel, M. Kohlhaas, E. Bertero, D. Wilhelm, M. Wagner, V. Sequeira, M. M. Kreusser, M. Dewenter, R. Kappl, M. Hoth, J. Dudek, J. Backs, C. Maack, CaMKII does not control mitochondrial Ca2+ uptake in cardiac myocytes, J. Physiol. 598 (2020) 1361–1376, https://doi.org/10.1113/JP276766.
[64] F. Fieni, D.E. Johnson, A. Hudmon, Y. Kirichok, Mitochondrial Ca2+ uniporter and CaMKII in heart, Nature 513 (2014) E1–E2, https://doi.org/10.1038/nature13626.
[65] H. Zhao, T. Li, K. Wang, F. Zhao, J. Chen, G. Xu, J. Zhao, T. Li, L. Chen, L. Li, Q. Xia, T. Zhou, H.Y. Li, A.L. Li, T. Finkel, X.M. Zhang, X. Pan, AMPK-mediated activation of MCU stimulates mitochondrial Ca2+ entry to promote mitotic progression, Nat. Cell Biol. 21 (2019) 476–486, https://doi.org/10.1038/s41556-019-0296-3.
[66] F. Zeng, X. Chen, W. Cui, W. Wen, F. Lu, X. Sun, D. Ma, Y. Yuan, Z. Li, N. Hou, H. Zhao, X. Bi, J. Zhao, J. Zhou, Y. Zhang, R.P. Xiao, J. Cai, X. Zhang, RIPK1 binds MCU to mediate induction of mitochondrial Ca2þ uptake and promotes colorectal oncogenesis, Cancer Res. 78 (2018) 2876–2885, https://doi.org/10.1158/0008-5472.CAN-17-3082.
[67] Z. Dong, S. Shanmughapriya, D. Tomar, N. Siddiqui, S. Lynch, N. Nemani, S. L. Breves, X. Zhang, A. Tripathi, P. Palaniappan, M.F. Riitano, A.M. Worth, A. Seelam, E. Carvalho, R. Subbiah, F. Jaña, J. Soboloff, Y. Peng, J.Y. Cheung, S. K. Joseph, J. Caplan, S. Rajan, P.B. Stathopulos, M. Madesh, Mitochondrial Ca2+ uniporter is a mitochondrial luminal redox sensor that augments MCU channel activity, Mol. Cell. 65 (2017) 1014–1028, https://doi.org/10.1016/j.molcel.2017.01.032, e7.
[68] S. Marchi, M. Corricelli, A. Branchini, V.A.M. Vitto, S. Missiroli, G. Morciano, M. Perrone, M. Ferrarese, C. Giorgi, M. Pinotti, L. Galluzzi, G. Kroemer, P. Pinton, Akt-mediated phosphorylation of MICU1 regulates mitochondrial Ca2+ levels and tumor growth, EMBO J. 38 (2019) e99435, https://doi.org/10.15252/embj.201899435.
[69] C.T. Madreiter-Sokolowski, C. Klec, W. Parichatikanond, S. Stryeck, B. Gottschalk, S. Pulido, R. Rost, E. Eroglu, N.A. Hofmann, A.I. Bondarenko, T. Madl, M. Waldeck-Weiermair, R. Malli, W.F. Graier, PRMT1-mediated methylation of MICU1

determines the UCP2/3 dependency of mitochondrial Ca2+ uptake in immortalized cells, Nat. Commun. 7 (2016) 12897, https://doi.org/10.1038/ncomms12897.

[70] J. Emerson, M.J. Clarke, W.L. Ying, D.R. Sanadi, The Component of "Ruthenium Red" Responsible for Inhibition of Mitochondrial Calcium Ion Transport. Spectra, Electrochemistry, and Aquation Kinetics. Crystal Structure of μ-O-[(HCO2)(NH3)4Ru]2Cl3, J. Am. Chem. Soc. 115 (1993) 11799–11805, https://doi.org/10.1021/ja00078a019.

[71] M.A. Matlib, Z. Zhou, S. Knight, S. Ahmed, K.M. Choi, J. Krause-Bauer, R. Phillips, R. Altschuld, Y. Katsube, N. Sperelakis, D.M. Bers, Oxygen-bridged dinuclear ruthenium amine complex specifically inhibits Ca2+ uptake into mitochondria in vitro and in situ in single cardiac myocytes, J. Biol. Chem. 273 (1998) 10223–10231, https://doi.org/10.1074/jbc.273.17.10223.

[72] J.J. Woods, N. Nemani, S. Shanmughapriya, A. Kumar, M. Zhang, S.R. Nathan, M. Thomas, E. Carvalho, K. Ramachandran, S. Srikantan, P.B. Stathopulos, J. J. Wilson, M. Madesh, A selective and cell-permeable mitochondrial calcium uniporter (MCU) inhibitor preserves mitochondrial bioenergetics after hypoxia/reoxygenation injury, ACS Cent. Sci. 5 (2019) 153–166, https://doi.org/10.1021/acscentsci.8b00773.

[73] J. Santo-Domingo, L. Vay, E. Hernández-SanMiguel, C.D. Lobatón, A. Moreno, M. Montero, J. Alvarez, The plasma membrane Na+/Ca2+ exchange inhibitor KB-R7943 is also a potent inhibitor of the mitochondrial Ca2+ uniporter, Br. J. Pharmacol. 151 (2007) 647–654, https://doi.org/10.1038/sj.bjp.0707260.

[74] N. Kon, M. Murakoshi, A. Isobe, K. Kagechika, N. Miyoshi, T. Nagayama, DS16570511 is a small-molecule inhibitor of the mitochondrial calcium uniporter, Cell Death Discov. 3 (2017) 17045, https://doi.org/10.1038/cddiscovery.2017.45.

[75] D.M. Arduino, J. Wettmarshausen, H. Vais, P. Navas-Navarro, Y. Cheng, A. Leimpek, Z. Ma, A. Delrio-Lorenzo, A. Giordano, C. Garcia-Perez, G. Médard, B. Kuster, J. García-Sancho, D. Mokranjac, J.K. Foskett, M.T. Alonso, F. Perocchi, Systematic identification of MCU modulators by orthogonal interspecies chemical screening, Mol. Cell 67 (2017) 711–723, https://doi.org/10.1016/j.molcel.2017.07.019, e7.

[76] G. Di Marco, F. Vallese, B. Jourde, C. Bergsdorf, M. Sturlese, A. De Mario, V. Techer-Etienne, D. Haasen, B. Oberhauser, S. Schleeger, G. Minetti, S. Moro, R. Rizzuto, D. De Stefani, M. Fornaro, C. Mammucari, A high-throughput screening identifies MICU1 targeting compounds, Cell Rep. 30 (2020) 2321–2331, https://doi.org/10.1016/j.celrep.2020.01.081, e6.

[77] A. De Mario, A. Tosatto, J.M. Hill, J. Kriston-Vizi, R. Ketteler, D. Vecellio Reane, G. Cortopassi, G. Szabadkai, R. Rizzuto, C. Mammucari, Identification and functional validation of FDA-approved positive and negative modulators of the mitochondrial calcium uniporter, Cell Rep. 35 (2021), 109275, https://doi.org/10.1016/j.celrep.2021.109275.

[78] C.V. Nicchitta, J.R. Williamson, Spermine a regulator of mitochondrial calcium cycling, J. Biol. Chem. 259 (21) (1984) 12978–12983, https://doi.org/10.1016/S0021-9258(18)90643-5.

[79] M. Montero, C.D. Lobaton, A. Moreno, J. Alvarez, A novel regulatory mechanism of the mitochondrial Ca2+ uniporter revealed by the p38 mitogen-activated protein kinase inhibitor SB202190, FASEB J. 16 (2002) 1955–1957, https://doi.org/10.1096/fj.02-0553fje.

[80] M. Montero, C.D. Lobatón, E. Hernández-Sanmiguel, J. Santodomingo, L. Vay, A. Moreno, J. Alvarez, Direct activation of the mitochondrial calcium uniporter by natural plant flavonoids, Biochem. J. 384 (2004) 19–24, https://doi.org/10.1042/BJ20040990.

[81] C.D. Lobatón, L. Vay, E. Hernández-SanMiguel, J. SantoDomingo, A. Moreno, M. Montero, J. Alvarez, Modulation of mitochondrial Ca 2+ uptake by estrogen receptor agonists and antagonists, Br. J. Pharmacol. 145 (2005) 862–871, https://doi.org/10.1038/sj.bjp.0706265.