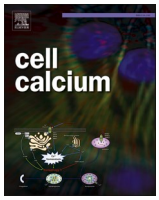
Cell Calcium 121 (2024) 102907

Contents lists available at ScienceDirect

Cell Calcium

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



Unravelling the complexity of the mitochondrial Ca^{2+} uniporter: regulation, tissue specificity, and physiological implications

Denis Vecellio Reane [a,*], Julian D.C. Serna [b], Anna Raffaello [c,*]

[a] Institute for Diabetes and Obesity, Helmholtz Diabetes Center (HDC), Helmholtz Zentrum Munich, Germany
[b] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil
[c] Department of Biomedical Sciences, University of Padova, Italy

ARTICLE INFO

Keywords:
Mitochondrial calcium uniporter
Calcium signalling
MCU regulation
tissue-specific modulation
Heart
Skeletal Muscle
Brain
Liver

ABSTRACT

Calcium (Ca^{2+}) signalling acts a pleiotropic message within the cell that is decoded by the mitochondria through a sophisticated ion channel known as the Mitochondrial Ca^{2+} Uniporter (MCU) complex. Under physiological conditions, mitochondrial Ca^{2+} signalling is crucial for coordinating cell activation with energy production. Conversely, in pathological scenarios, it can determine the fine balance between cell survival and death. Over the last decade, significant progress has been made in understanding the molecular bases of mitochondrial Ca^{2+} signalling. This began with the elucidation of the MCU channel components and extended to the elucidation of the mechanisms that regulate its activity. Additionally, increasing evidence suggests molecular mechanisms allowing tissue-specific modulation of the MCU complex, tailoring channel activity to the specific needs of different tissues or cell types. This review aims to explore the latest evidence elucidating the regulation of the MCU complex, the molecular factors controlling the tissue-specific properties of the channel, and the physiological and pathological implications of mitochondrial Ca^{2+} signalling in different tissues.

1. Introduction

The foundation of mitochondrial Ca^{2+} signalling can be traced back to the 1960s with pioneering studies demonstrating the active uptake of Ca^{2+} by energized isolated mitochondria [1,2]. Despite significant insights into the regulation and diverse functions of mitochondrial Ca^{2+} signalling, including its role in aerobic metabolism and cell survival [3,4] (reviewed in [5–7]), the identity of the channel responsible remained elusive for a long time. This limitation hindered further research aimed at better understanding the physiological role of mitochondrial Ca^{2+} signalling in the organism and tissue homeostasis. Two breakthrough discoveries since 2010, the elucidation of the molecular identity of the mitochondrial Ca^{2+} uniporter regulator MICU1 [8] and of MCU itself [9,10], marked the beginning of the molecular era in mitochondrial Ca^{2+} research.

The identification of MCU marked a turning point in understanding mitochondrial Ca^{2+} signalling. Following the discovery of MCU, additional components were progressively identified in the years that followed. This progress has significantly deepened our understanding of the functional regulation of the MCU complex. Despite this advancement in identifying the components of the channel and the recent elucidation of its complete protein architecture (reviewed in [11–13]), several key questions remain regarding its precise modulation in different tissues. These questions include how the channel is modulated under physiological conditions and during the progression of pathological states. Further research is needed to elucidate these aspects.

Unanswered questions that require future investigation include how changes in the expression, assembly and stability of individual elements affect channel activity, and how this is regulated in different tissue types and under different physiopathological conditions. This review aims to present recent findings on the mechanisms of regulation of MCU channel and insights into the role of mitochondrial Ca^{2+} signalling in different organs, emphasising the specific molecular properties in the different tissues and cell types.

2. The MCU complex

The membrane-spanning subunits of the MCU complex are composed of three different proteins: MCU, MCUb and EMRE [9,10,14,15]. The MCU spans the inner mitochondrial membrane (IMM) with a pair of transmembrane domains connected by a short loop oriented towards the intermembrane space (IMS) [16]. Consequently, both the N- and

* Corresponding authors.
E-mail addresses: denis.vecellioreane@helmholtz-munich.de (D. Vecellio Reane), anna.raffaello@unipd.it (A. Raffaello).

https://doi.org/10.1016/j.ceca.2024.102907
Received 23 April 2024; Received in revised form 10 May 2024; Accepted 13 May 2024
Available online 23 May 2024

C-terminal domains reside within the mitochondrial matrix. Functionally, the MCU must self-assemble into tetramers to form the pore of the channel [16,17]. A key element in this assembly is the DIME motif, a four-amino-acid stretch between the second transmembrane domain and the loop. This motif oligomerises with radial symmetry, creating a negatively charged region at the IMS opening of the channel, forming the selectivity filter for the channel [16]. On the other side of the IMM, the N-terminal domain of MCU can interact extensively with another MCU tetramer, forming a v-shaped dimer. This suggests that dimerization may be involved in a specific localisation of the MCU complex within specific subdomain of the mitochondrial IMM [16] (see below).

Despite the ability of the MCU tetramer to oligomerise in this channel architecture, MCU in metazoans remains unable to facilitate *in vivo* Ca^{2+} permeation [18]. Indeed, effective channel function requires the presence of the 10 kDa protein EMRE [14]. Structurally, EMRE consists of a single transmembrane domain together with short N- and C-terminal domains. The IMS-oriented N-terminal domain contains a polybasic sequence of amino acids relevant for interacting with regulatory subunits [16]. On the other hand, insights from the protein structure of the MCU complex show that the C-terminal domain of EMRE is essential for maintaining the MCU channel in a functional configuration that allows Ca^{2+} permeation [19].

Within the channel, MCU subunits can be replaced by one or more auxiliary subunits MCUb [15]. MCUb shares a significant homology with the MCU primary sequence, particularly in the highly conserved transmembrane and loop domains. However, there are specific amino acid substitutions in the loop region of MCUb. These substitutions critically affect the ability of the MCU complex to allow Ca^{2+} permeation when MCUb is incorporated into the complex [15]. Additionally, recent molecular modelling of MCUb, based on the molecular structure of the MCU complex, suggests that incorporating MCUb can alter the overall structure of the complex. These changes include a reduction in the interfacial interaction between MCUb and EMRE, as well as a change in the pore size of the complex [20]. While the N-terminal domain of MCUb retains substantial homology with MCU, specific amino acid substitutions may potentially account for differences in the dimerization properties of MCU complexes containing MCUb moieties.

The regulatory components of the channel are represented by the MICU proteins - MICU1, MICU2 and MICU3. These proteins reside in the IMS and contain two EF-hand Ca^{2+}-binding motifs in their protein structure, allowing them to sense changes in Ca^{2+} concentrations ($[Ca^{2+}]$). MICU1 is the central regulatory component of the MCU complex [21,22]. It forms dimers stabilised by disulfide bonds linking conserved cysteines within its C-terminal domains, both with MICU1 itself and with either MICU2 or MICU3 [23–25]. This MICU dimer interacts with the MCU complex through the IMS pore aperture and the polyaspartate tail of EMRE. The initial interaction involves a lysine/arginine ring (K126, R129, R259, R261 and R263) and the D261 ring

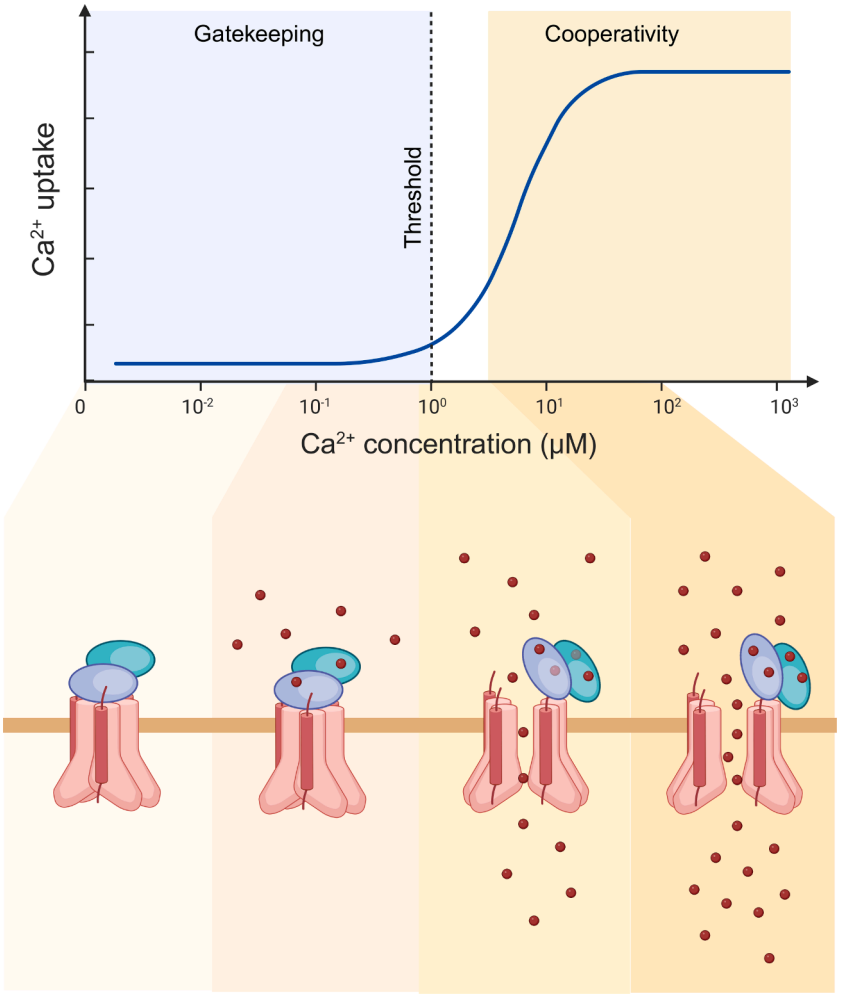


Fig. 1. The sigmoidal activity of the MCU complex. (A) Graph illustrating the relationship between $[Ca^{2+}]_{cyt}$ and mitochondrial Ca^{2+} uptake rate. At low $[Ca^{2+}]_{cyt}$, the MICUs dimers inhibit mitochondrial Ca^{2+} uptake until a specific threshold is reached. Beyond this threshold, cooperative activation occurs, leading to a steep increase until maximal capacity is reached. (B) MICUs dimer acts as a gatekeeper at lower $[Ca^{2+}]_{cyt}$, releasing channel inhibition. Continued elevation of $[Ca^{2+}]_{cyt}$ saturates the EF-hand Ca^{2+} binding domains, prompting cooperative channel activation.

at the MCU pore [16]. The interaction with the polybasic region of MICU1 and the polyaspartate tail of EMRE keeps the dimer in proximity to the MCU complex, even when gatekeeping is relieved [16]. The MICU dimer exerts pivotal regulatory functions in regulating channel activity. In resting cells, it keeps the channel in a closed state, preventing excessive mitochondrial Ca^{2+} influx and Ca^{2+} vicious cycles. When Ca^{2+} concentrations escalate, the gatekeeping mechanism is relieved and MICU orchestrates cooperative channel activation, allowing for a rapid mitochondrial response to Ca^{2+} influx [22,26,27].

3. Regulation of the channel activity

For several decades, numerous reports have consistently shown a sigmoidal relationship between cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) and the rate of mitochondrial Ca^{2+} uptake ([28] and Fig. 1). At low $[Ca^{2+}]$, the rate of Ca^{2+} uptake by mitochondria is notably inhibited until a specific threshold is reached. Beyond this point, mitochondrial Ca^{2+} uptake exhibits a cooperative activation, leading to a sharp increase in the uptake rate, until it reaches a maximum capacity [22]. Indeed, the sigmoidal activity of the MCU complex serves as an adaptive mechanism. It allows mitochondria to filter out minor increases in $[Ca^{2+}]_{cyt}$, preventing chronic and unnecessary stimulation. At the same time, it enables mitochondria to respond efficiently to significant and rapid spikes or oscillations in $[Ca^{2+}]_{cyt}$ during periods of sustained cellular activity. Nevertheless, it is important to note that changes in the activation threshold and the extent of cooperative activation can significantly influence how mitochondria respond to Ca^{2+} challenges across different tissues [26]. This allows mitochondria to adapt to the specific needs and demands of each context. Traditionally, these distinctive properties have been attributed to the MICUs dimers [22,23,25], which are the only subunits known to sense $[Ca^{2+}]$. However, the intricate molecular composition of the MCU complex introduces an intriguing possibility of potential regulatory layers. New insights into these potential layers are needed for a more complete understanding.

3.1. Membrane spanning subunits

Recent advancements in our understanding of the MCU complex have revealed a complex interplay of regulatory mechanisms within the membrane spanning subunits of the channel. First, studies using patch clamp recordings showed that MCU current density is directly influenced by the concentration of Ca^{2+} in the mitochondrial matrix ($[Ca^{2+}]_{mit}$) [29]. At normal resting matrix Ca^{2+} levels, there is significant inhibition of MCU activity, with maximal channel inhibition occurring at approximately 400 nM of $[Ca^{2+}]_{mit}$ [30]. This suggests that the matrix $[Ca^{2+}]$ is a key regulator of MCU activity. The proposed matrix Ca^{2+} sensor appears to be linked to the ability of the MCU N-terminal domain to bind cations with low affinity [31]. Mutations in critical residues within this domain has been shown to abrogate the inhibitory effect of $[Ca^{2+}]_{mit}$ on MCU activity [29]. From a physiological perspective, this mechanism is thought to serve as a protective mechanism against mitochondrial Ca^{2+} overload. However, it is important to note that the regulation of MCU activity by $[Ca^{2+}]_{mit}$ depends on the association of MICU1/2 with the channel on the opposite side of the IMM [29]. This suggests a tight coupling between $[Ca^{2+}]_{mit}$ and MICU1/2 in regulating MCU activity. As a result, dissecting the precise mechanism of $[Ca^{2+}]_{mit}$ regulation of MCU remains challenging, and concrete evidence of its physiological and pathological relevance is still awaited, as most of the studies conducted so far have been conducted in cells.

A secondary level of regulation within the MCU complex involves the integration of MCUb into the channel. MCUb can replace any subunit of MCU within the tetrameric complex, resulting in a reduction in the Ca^{2+} conductance of the MCU complex. Indeed, substitution of two conserved residues near the DIME motif has been shown to significantly affect channel conductance [15]. There is a general consensus that considers MCUb a dominant-negative regulator of MCU channel activity [15,32,33]. The precise extent to which the substitution of MCU for MCUb in the stoichiometry of the MCU complex affects the channel conductance remains somewhat unclear. Nevertheless, insights from studies involving MCU knockout (KO) mouse models suggest that MCUb tetramers are unable to conduct Ca^{2+}. This is evident from the fact that MCU depletion alone is sufficient to completely abolish mitochondrial Ca^{2+} influx into the mitochondrial matrix [34]. Furthermore, recent evidence supports the notion that MCUb diminishes the functionality of the channel. This evidence shows that MCUb is unable to interact with EMRE [33] and/or MICUs [32]. This lack of interaction can be attributed to both a decreased presence of EMRE within the complex and a reduction in the cooperative activation of the channel activity due to the loss of MICU1 interaction.

3.2. Regulatory subunits

The MICUs represent the most extensively studied regulators of the MCU complex, primarily due to their canonical EF-hand Ca^{2+}-binding domains [35]. These subunits exert control over the activation threshold and cooperative activation of the channel, as demonstrated in previous studies [22,23,25,36]. These parameters are crucial in determining the amplitude of the mitochondrial Ca^{2+} response. Indeed, variations in the activation threshold determine the minimal stimulus required to trigger mitochondrial Ca^{2+} uptake and influence the speed at which mitochondria respond to cellular activation [26,27]. Similarly, different levels of cooperative channel activation confer different Ca^{2+} uptake capacities to mitochondria facing identical challenges (Fig. 2). MICU1 was originally identified as the gatekeeper and cooperative activator of the channel. Indeed, KO of MICU1 in cell cultures not only eliminates the activation threshold, but also reduces the cooperative activation ([22] and Fig. 2A). The subsequent discovery of the paralogous MICU2 and MICU3 [35], coupled with the revelation of the dimeric nature of MICUs, further complicates the regulatory landscape [23,25]. It is worth noting that MICU1 is the subunit that directly interacts with the channel [16]. As a consequence, the MICUs dimer always includes MICU1 [23,25]. In this configuration, MICU1 can form dimers with itself, resulting in MICU1 homodimers, or with one of the other MICUs, MICU2 and MICU3, leading to the formation of different MICUs heterodimers [23–25]. Generally, these dimers inhibit the channel when not bound to Ca^{2+}, and this inhibition is relieved by Ca^{2+} binding to their EF-hand domains. The Ca^{2+} affinity of the MICUs dimers is crucial in governing this process. The consensus is that the MICU1:MICU1 homodimer shows a higher Ca^{2+} affinity compared to the MICU1:MICU2 dimer ([37] and Fig.s 2B-C). Although the Ca^{2+} affinity of the MICU1:MICU3 dimer has not been measured yet, functional analyses of MICU3 suggest a strong activation effect, indicating a potentially higher affinity compared to the MICU1:MICU1 homodimer ([24,38] and Fig. 2D). The specific functional role of Ca^{2+} binding to the EF-hand domains of one monomer versus the other in the regulation of the activation threshold and cooperative activation is still under investigation. However, for the MICU1:MICU2 dimer, it is clear that Ca^{2+} must bind to both the MICU1 and MICU2 EF-hand domains to fully activate MCU-dependent mitochondrial Ca^{2+} uptake [23,25].

3.3. Tissue-expression of the MCU complex components

The composition and regulation of the MCU complex provides multiple levels of modulation of channel properties. One of the more straightforward means of modulation is through the differential expression of the channel components, namely MCU, MCUb, and EMRE. This concept is supported by various lines of evidence correlating MCU current density in different tissues through electrophysiological measurements [39] with the varying expression levels of MCU [9], EMRE [14], and MCUb [15]. MCUb is particularly interesting in this context, as it shows low expression levels in most tissues [15]. The MCU:MCUb ratio

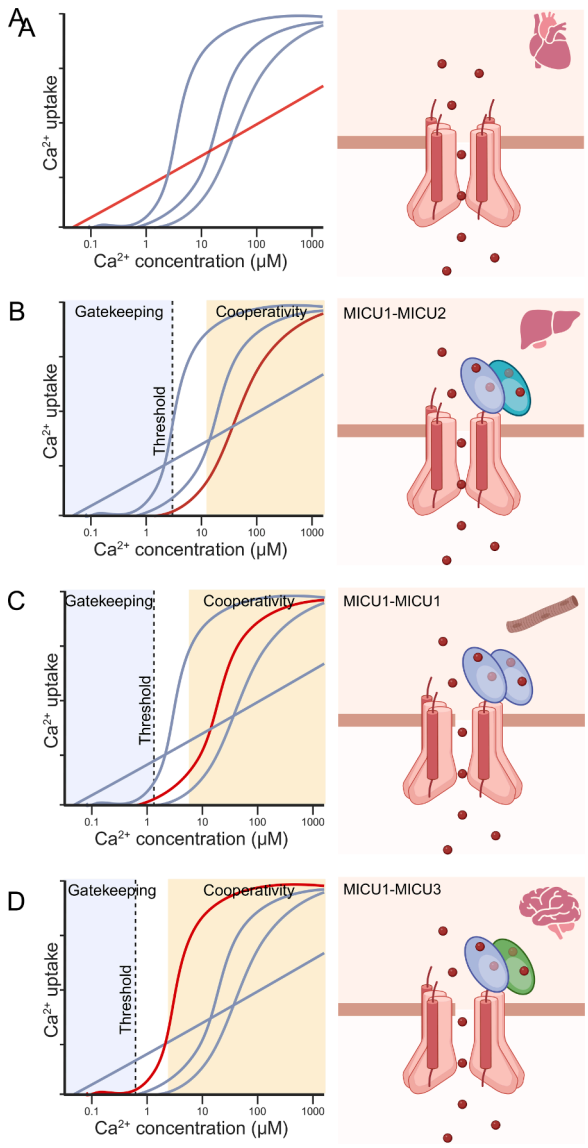


Fig. 2. Alteration of the threshold and cooperative activation of the MCU complex contingently to the different expression of the MICUs dimers. In cardiac tissues (A) absence of the MICUs dimer eliminates the threshold and cooperative activation. MICU1:MICU2 heterodimer (B) establishes a high threshold and strong cooperative activation of the channel, decoding cytosolic Ca^{2+} transients into $[Ca^{2+}]_{mit}$ increases as seen in hepatic tissue. MICU1 homodimers (C) in skeletal myotubes or MICU1:MICU3 heterodimers (D) in brain neurons decrease activation threshold and facilitates a more immediate cooperative activation of the complex.

becomes crucial when studying tissue-specific regulation of the channel. For example, the heart stands out as an important example, where the MCU:MCUb ratio is ten times lower compared to skeletal muscle. Not surprisingly, this results in heart mitochondria having ten times lower MCU current density than skeletal muscle mitochondria [39]. A fascinating aspect is that recent reports have demonstrated that MCUb expression can be modulated during various physiological and pathological conditions. For instance, changes in MCUb expression have been observed during the maturation of macrophages from a pro-inflammatory to an anti-inflammatory phenotype in experimental models of muscle damage and regeneration ([40] and Fig. 3A). In MCUb knockout ($MCUb^{KO}$) mice, the switch in macrophage phenotype is impaired, affecting muscle repair after injury [40]. Furthermore, MCUb induction during fasting in skeletal muscle has been observed, influencing substrate preference by enhancing fatty-acid utilisation ([41] and Fig. 3B). In the cardiac context, MCUb expression significantly rises post-myocardial infarction ([32,33] and Fig. 3C). The role of MCUb in the heart has been further explored in a mouse model of type 2 diabetic cardiomyopathy [42], where MCUb levels are upregulated as a stress-responsive mechanism to limit Ca^{2+} overload during cardiac injury.

In addition to the membrane-spanning subunits of the channel, the regulatory subunits MICUs play a pivotal role, and differential expression levels of these paralogous proteins can dramatically alter the activation threshold and the cooperative activation of the channel. Recent research has highlighted the heterogeneity of the MICUs dimers in different tissues [24,26,27,38,43]. One striking example of tissue-specific MICU regulation can be found in skeletal muscle. Here, a tissue-specific splicing variant of MICU1 is expressed, and the addition of a single exon containing four amino acids significantly increases the affinity of the MICUs dimer for Ca^{2+}, shifting the threshold for MCU opening to lower Ca^{2+} concentrations [43]. This adaptation enables a rapid response of mitochondrial oxidative metabolism to skeletal muscle activity, ensuring an efficient supply of ATP for contraction. Intriguingly, this splicing event is tightly regulated by specific splicing factors whose expression increases during muscle differentiation [44]. Another tissue-specific MICU is MICU3, which was originally characterised as a neuronal-specific regulator of MCU, but nowadays identified as player in mitochondrial Ca^{2+} homeostasis in different other tissues like heart and skeletal muscle [45,46]. Like MICU1.1, MICU3 can enhance mitochondrial Ca^{2+} signalling through MCU when incorporated into the complex [24,38]. Recent investigations have revealed a general trend in electrically excitable cells where MCU channels appear to be configured for easier activation [26,27]. In skeletal muscle, for example, there is a predominant expression of MICU1 homodimers. In neurons, there is expression of MICU1:MICU3 dimers, and cardiomyocytes have very low levels of MICUs overall. This specific configuration of the MCU complex in these excitable cells allows for a rapid response of mitochondrial metabolism to cell activation, enabling the efficient production of ATP to support energy-demanding processes such as neurotransmission and muscle contraction. In contrast, hepatocytes control the MCU channel through the MICU1:MICU2 heterodimer, which provides a robust activation threshold and strong cooperative channel activation. The differential expression of MICUs is particularly important as it can modify the activation properties of the channel by altering the relative expression of the different MICUs and promoting the formation of different MICUs dimers [26,27]. While MICU proteins are essential for mitochondria to decode cytoplasmic Ca^{2+} signals under normal conditions, several studies demonstrate that their protein expression can change under both physiological and pathological conditions [47–50]. For example, a decrease in MICU2 expression has been observed in the liver during fasting. This change could lead to a shift from MICU1:MICU2 heterodimers to MICU1 homodimers, resulting in an increased rate of Ca^{2+} uptake and ATP production [47,48]. Similar adaptations during fasting have been observed in renal pathological conditions, such as in a model of autosomal dominant polycystic kidney disease [51].

3.4. *MCU complex post-translational modifications*

Another layer of complexity arises from the post-translational modifications (PTMs) undergone by various components of the MCU channel, which significantly influence their activity. Phosphorylation, in particular, has been extensively studied. Initial investigations in 2012 identified phosphorylation of MCU Ser92 by CAMKII, supposedly activating MCU [52]. However, subsequent patch-clamp experiments and $CaMKII^{KO}$ animal models have raised doubts about this finding [53,54]. Another notable phosphorylation event occurs at Tyr289 by proline-rich tyrosine kinase 2 (Pyk2), which promotes channel oligomerization and enhances mitochondrial Ca^{2+} signalling [55]. This modification may have pathological implications, as activation of the MCU/Pyk2 pathway in brain ischemia stimulates MCU channel activity, leading to

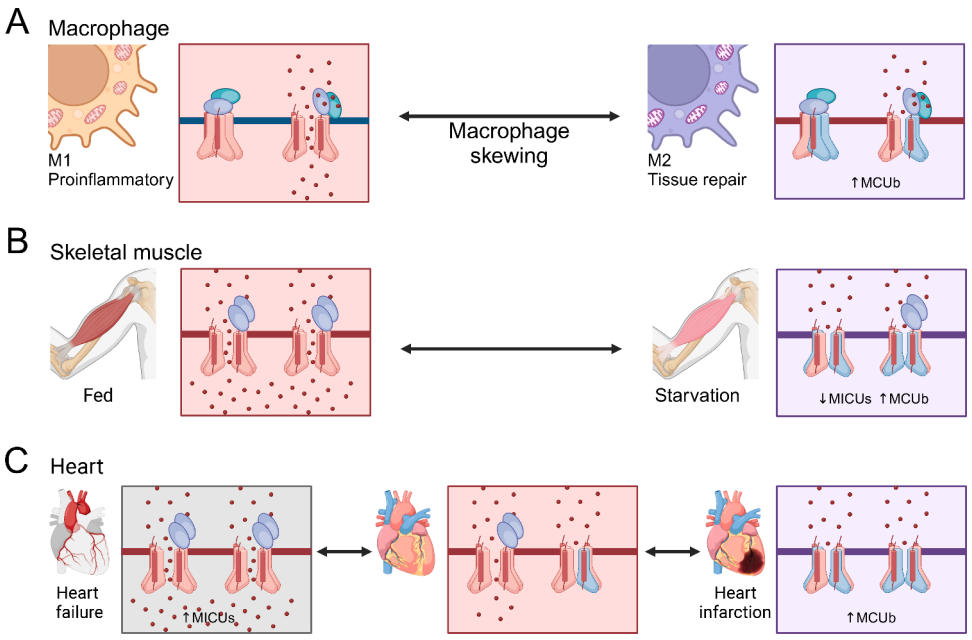


Fig 3. Remodelling of the MCU complex in physiological and pathological conditions. (A) Transition from proinflammatory to anti-inflammatory macrophages involves elevated MCUb expression, regulating macrophage metabolism towards a reparative phenotype. (B) In skeletal muscle, fed and calorie-restricted states induced by fasting upregulate MCUb expression, initiating mitochondrial metabolic adaptation favouring fatty acid utilization for energy production. (C) Post-myocardial infarction, elevated MCUb expression decreases mitochondrial Ca^{2+} uptake, limiting Ca^{2+} overload. However, heart failure exhibits increased MICU1 expression, impacting mitochondrial Ca^{2+} dynamics and channel gating, potentially affecting energy metabolism and contractile dysfunction.

detrimental effects on mitochondrial function and neuronal survival [56]. Additionally, AMPK-mediated phosphorylation of Ser57 on MCU can positively modulate mitochondrial Ca^{2+} entry, enhancing mitochondrial respiration and energy production, which are critical for cell replication [57].

PTMs, especially phosphorylation, have been extensively studied in cancer cell lines, revealing their involvement in tumour progression and malignancy. For example, phosphorylation of MCU has been implicated in the development of colorectal cancer (CRC). In CRC, receptor-interacting protein kinase 1 (RIPK1) interacts with MCU to enhance mitochondrial Ca^{2+} uptake and energy metabolism, thereby promoting CRC cell proliferation. Additionally, Akt kinase-mediated phosphorylation of MICU1 at Ser124 disrupts the MICU1-MICU2 dimer, inhibiting MICU1's suppressive effect on MCU. [58]. This leads to elevated basal mitochondrial matrix Ca^{2+} levels, abnormal reactive oxygen species (ROS) production, and tumour progression [58]. Accordingly, mutants of MICU1 that cannot be phosphorylated restore normal mitochondrial Ca^{2+} and ROS levels, thereby inhibiting Akt-driven tumour growth [58].

In addition to phosphorylation, other PTMs can also influence MCU channel activity. One such modification is MCU oxidation, particularly at Cys97. Upon oxidation, Cys97 undergoes reversible S-glutathionylation, which promotes the assembly of MCU channels into higher-order complexes with sustained activity, resulting in increased mitochondrial Ca^{2+} accumulation [59]. Moreover, MCU oxidation can also occur through interaction with Complex I of the electron transport chain, leading to uniporter degradation. However, under conditions of Complex I dysfunction, this interaction is lost and MCU levels increase, thereby sustaining mitochondrial Ca^{2+} levels to preserve bioenergetic homeostasis [60]. Additionally, post-translational methylation of MICU1 by arginine methyltransferase 1 (PRMT1) at Arg455 reduces its sensitivity to Ca^{2+}[61]. UCP2/3 can restore the Ca^{2+} sensitivity of methylated MICU1, thereby reinstating mitochondrial Ca^{2+} uptake activity [61–63].

3.5. MCU complex proteolytic processing and assembly

The MCU complex, with its numerous subunits, is assembled in different configurations that are influenced by the expression levels of its components. Additionally, the process is regulated by specific mitochondrial proteases and chaperones (Fig. 4). For example, the integration of EMRE into the channel occurs after its assembly with MICUs dimers, a sequential process designed to prevent the formation of ungated channels [64]. Interestingly, mAAA-type proteases exhibit the ability to discriminate between uncomplexed and MCU-bound forms of EMRE, selectively degrading the former (as explained below). This selective degradation promotes the balanced expression of MCU and EMRE, which is essential for the proper assembly and function of the channel complex [65]. The mitochondrial peptidase MAIP1 acts as a chaperone during the assembly of EMRE with MCU [64]. This mechanism has particulars pathological relevance, as the loss of the m-AAA protease leads to the accumulation of constitutively active MCU-EMRE channels lacking gatekeeper subunits. This accumulation can particularly affect neuronal mitochondria, leading to mitochondrial Ca^{2+} overload [64]. The number of EMRE units required for a functional channel is still unclear. While the cryo-EM structure of the MCU complex proposed a 1:1 ratio between MCU and EMRE proteins [16,19], it remains unclear whether complexes with fewer than four EMREs can still permeate Ca^{2+}, and how the properties of the complex change under these conditions. Evidence suggests that altered MCU:EMRE stoichiometry in cells leads to the formation of an active channel but fails to properly tether MICUs dimers to the complex, resulting in altered gatekeeping and cooperative activation profiles [66]. Furthermore, the elucidation of the protein structure of the MCU complex has introduced another potential level of regulation in its assembly. The cryo-electron microscopy (cryo-EM) structure of the MCU complex, determined at high [Ca^{2+}], reveals the formation of a dimer of the holocomplex [16, 67]. The authors of this discovery speculate that this dimeric configuration may favour the localisation of the channel to the most superficial IMM rather than to the invaginated cristae regions. Consequently, this could play a pivotal role in regulating mitochondrial Ca^{2+} uptake

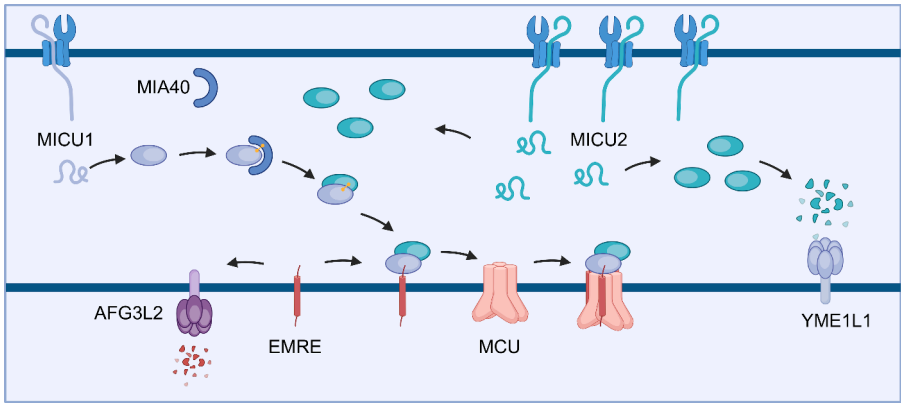


Fig. 4. Assembling and proteolytic processing of the MCU complex components. (A) EMRE integrates into the channel after associating with MICUs dimers, preventing uncontrolled channel formation. Excessive EMRE levels may be degraded by the m-AAA protease AFG2L3, contributing to regulatory control. (B) MICUs assembly involves a two-step process. Initially, the IMS oxidoreductase Mia40 catalyzes disulfide bond formation in MICU1. The oxidized MICU1 then interacts with another MICUs protein to form the dimeric structure. (C) The IMS AAA-protease YME1L1 regulates MICUs protein levels by selectively degrading MICUs monomers not engaged in dimeric states stabilized by disulfide bonds, aiding in maintaining proper assembly of MICUs dimers within the mitochondrial calcium uniporter complex.

through the precise localization of MCU complexes to specific regions within the IMM [16]. Other factors can influence the oligomerization of MCU channels, such as the previously mentioned S-glutathionylation of the NTD domain of MCU [59]. Another mechanism involves the mitochondrial protein MCUR1, initially identified as a regulator of MCU [68,69] and more recently suggested to act as a cytochrome c oxidase assembly factor [70]. Recently it has been shown that the deletion of MCUR1 affect also the MCU oligomeric state, impacting on the MCU channel activity [71].

Recent findings challenge the traditional view that the MCU complex remains in a stable configuration until activated by increased [Ca^{2+}] in the IMS [72,73]. It has been demonstrated that MICU1 localizes to the inner boundary membrane (IBM) of the IMM in a manner dependent on mitochondrial membrane potential, where it contributes to stabilizing the cristae junctions [72,74], through direct interactions with MIC60 and CHCHD2 [74]. Furthermore, recent investigations suggest that upon Ca^{2+} mobilization, MICU1 anchors MCU/EMRE at the IBM, proposing a "potentiation mechanism" in which MICU1 enhances channel activity upon stimulation rather than blocking it in its resting state [73], supported by some evidence from patch clamp recordings [67]. However, the validity of this potentiation mechanism is still debated in the field [75–77], although these recent discoveries will undoubtedly stimulate further investigations into the regulatory properties of the MCU channel.

The regulatory subunits of the MCU complex can undergo dynamic assembly both with themselves and with the channel. For example, it has been shown that an increase in $[Ca^{2+}]_{cyt}$ leads to the rearrangement of MICU1 multimers into smaller complexes in intact cells [78]. This rearrangement could potentially alter the regulatory profile of MICU1. Furthermore, few reports have addressed the assembly of the regulatory subunits, MICUs. However, it appears to be a two-step mechanism. First, MICUs form dimers stabilized through the creation of a disulfide bond, a process catalysed by the IMS oxidoreductase Mia40. This enzyme oxidises conserved cysteines within the MICUs to create mature dimers [79]. Initially, the disulfide bond was thought to be essential for the proper MICU function. However, recent research has shown that MICUs can dimerise even in the absence of disulfide bond formation [27]. In fact, mutating the cysteine involved in the disulfide bridge in MICU1 does not prevent MICU1 from interacting with the channel or effectively regulating channel activity [27].

An intriguing aspect is the regulation of the formation of different MICUs dimers. Some tissues have both MICU1:MICU2 dimers and MICU1 homodimers, whereas MICU2 homodimers have never been observed *in vivo*. This seems to be because MICU1 has a high affinity for itself, favouring homodimerization when other MICU1 monomers are present. To prevent this and to facilitate the formation of heterodimers, the import of MICU1 into the IMS must be tightly controlled, allowing for the accumulation of MICU2 through a faster import process [27]. The precise molecular mechanisms controlling these differential import rates remain unknown. However, when MICU1 import increases or MICU2 abundance decreases, MICU1 readily encounters other MICU1 molecules and forms homodimers. Recently, it has been suggested that unlike MICU1, MICU2 lacks a specific Mia40 recognition sequence. This might explain of the absence of MICU2 homodimers, possibly due to their faster degradation [27].

3.6. *Proteolytic processing and degradation of MCU complex.*

In recent years, the regulation of proteolytic degradation of the MCU complex components has emerged as a critical aspect of mitochondrial Ca^{2+} homeostasis. Precisely control of the levels of these components is essential for proper assembly and physiological control of channel activity (Fig. 4). While the regulation of proteolytic degradation of MCU itself remains poorly understood, MCU appears to be a highly stable protein with a long half-life [80]. In contrast, other MCU complex components such as EMRE (with a half-life of about 8 hours) [65] and MICU1 (with a half-life of about 4 hours) [27] have shorter lifespans. This is where the mitochondrial protein quality control system comes into play, particularly through the action of matrix and IMS AAA-proteases that regulate the degradation of various MCU complex components. For instance, excessive EMRE is targeted for degradation by m-AAA-proteases like AFG2L3 and SPG7. This regulatory process prevents the over-incorporation of EMRE into the complex, which could lead to the formation of uncontrolled MCU channels [64,65]. The mitochondrial protein quality control system also plays a role in the controlled degradation of the regulatory subunit MICUs. YME1L1, an IMS AAA-protease, regulates MICUs protein levels. Recent findings suggest that YME1L1 efficiently degrades MICUs monomers when they are not in a disulfide-stabilised dimeric state [27]. This mechanism appears to enhance the stability of MICUs and explains why they are typically detected as dimers under non-reducing conditions. Notably, MICU2, which lacks Mia40 recognition sequence and does not form homodimers through disulfide bond stabilization, is rapidly degraded, preventing the accumulation of this particular dimeric form [27]. These regulatory processes ensure precise control over the levels and forms of MCU complex components, contributing to the fine-tuned regulation of mitochondrial Ca^{2+} uptake.

4. The physiological roles of the Mitochondrial Ca^{2+} Uniporter

Understanding the tissue-specific regulation of mitochondrial Ca^{2+} signalling does not mean that its role in tissue homeostasis has been fully elucidated. To unravel this importance, researchers have employed various animal models, including those with genetic modifications of the MCU complex, to elucidate its physiological role during embryonic development and in tissue homeostasis.

4.1. Germline deletion of MCU channel components

The most extensively characterised MCU channel components in the context of animal models is MCU itself. Researchers have generated models in *C. elegans, D. melanogaster, Zebrafish* and mice [34,81–85]. The absence of MCU is surprisingly well tolerated by several organisms. However, analysis of the total MCU KO mice (MCU^{KO}) reveals a more complex picture. While MCU^{KO} mice from the outbred CD1 strain are viable, approximately half of the offspring die during development. Viable outbred CD1 MCU^{KO} mice exhibit smaller size, reduced organ and body weight (with preserved organ/body weight ratio), altered pyruvate dehydrogenase (PDH) phosphorylation and activity, and a marked impairment in their ability to perform strenuous work [34]. Interestingly, total MCU^{KO} is embryonically lethal in the inbred C57BL/6 strain, which dies before E14.5 of embryonic development [34,57]. These findings suggest a background-dependent compensatory mechanism that emerges to overcome the deletion of MCU, particularly when the genetic diversity allows for adaptative flexibility. Studies conducted in cell lines further support the importance of MCU and mitochondrial Ca^{2+} uptake during cell cycle progression. MCU is required to couple mitochondrial functions to metabolic demands during the G1-S transition and mitosis of the cell cycle [86]. The production of new cells imposes significant energetic and biosynthetic demands on the mother cell to ensure proper progression throughout the cell cycle [87,88]. Therefore, MCU^{KO} mice exhibit delayed wound healing, reduced numbers of proliferating cells during liver regeneration, and fewer cells in organs with limited postnatal proliferation [86]. Similarly, *C. elegans* lacking MCU shows defective wound healing and motility [84,89]. Another critical MCU component analysed in mouse models is EMRE. Given its essential role in enabling the MCU channel to Ca^{2+} permeation, total EMRE KO mouse ($EMRE^{KO}$) has a phenotype similar to total MCU^{KO} mouse. Mouse models with EMRE deletion reveal that mitochondria are unable to rapidly take up Ca^{2+} [90]. In an outbred background, a fraction of $EMRE^{KO}$ mice is born and appears healthy and active, albeit smaller than wild-type (WT) littermates. $EMRE^{KO}$ mouse has unaltered basal metabolism and, unlike MCU^{KO} mouse, is not significantly impaired in exercise capacity [90]. In the context of the regulatory subunits, mouse models with total KO for MICU1 and MICU2 have been generated ($MICU1^{KO}$ and $MICU2^{KO}$). Initially, it was observed that the whole-body knockout of MICU1 leads to lethality within hours after birth, with $MICU1^{KO}$ newborns displaying a failure of basic vital functions after-birth [91]. However, a subsequent mouse model reported a perinatal lethality rate of approximately 85%, with the surviving $MICU1^{KO}$ mice exhibiting ataxia and muscle weakness [92]. This phenotype resemble that of human patients with loss-of-function mutations in MICU1 [93]. Interestingly, as the $MICU1^{KO}$ mice age their phenotype appears to improve and they regain near-normal mitochondrial Ca^{2+} homeostasis, coinciding with a decrease in EMRE protein expression [92].

Conversely, $MICU2^{KO}$ mice are viable and produce offspring in Mendelian ratios, with comparable sizes and activity levels to their wild-type littermates. Consistent with previous results using RNAi targeting MICU2 in mouse liver [35], both MICU1 and MCU protein levels were significantly reduced in liver tissue from $MICU2^{KO}$ mice. Deletion of MICU2 leads to more rapid mitochondrial Ca^{2+} uptake in response to low cytosolic Ca^{2+} challenges, while overall Ca^{2+} uptake is reduced upon greater stimulation, likely due to a reduction in MCU levels [94].

4.2. Heart

The rhythmic contraction and relaxation of the heart muscle are essential to drive blood circulation and ensure adequate organ perfusion. This dynamic process places high energy demands on cardiomyocytes, which are met primarily by oxidative phosphorylation. It is therefore not surprising that mitochondria, which constitute approximately 30% of cardiomyocyte volume, play a pivotal role in cardiac physiology and pathology. Heart performance and mitochondrial activity are finely tuned to the needs of the body [95,96]. This occurs in the fight-or-flight response, where heart rate, contractile force, and mitochondrial metabolism increase following β-adrenergic stimulation [97].

Ca^{2+} is central to excitation-contraction coupling, acting as a permissive factor for cross-bridge cycling and coupling energy metabolism to cardiomyocytes activation [96,97]. Under pathological conditions, mitochondrial Ca^{2+} overload is a major cause of cardiomyocyte cell death, primarily through the Ca^{2+}-induced mitochondrial permeability transition (mPT) during ischemia/reperfusion (I/R) injury [4, 98–100]. While several lines of evidence support the role of $[Ca^{2+}]_m$ in fine-tuning cardiomyocyte bioenergetics [95,96], the precise contributions of the MCU complex to cardiac physiology and pathology are less well understood. For this reason, cardiac functionality and the response to physiological or pathological stimuli were evaluated across various mouse models with affected $[Ca^{2+}]_m$ (Table 1). These models included global KO of MCU, MCUb and EMRE (MCU^{KO} [34], $MCUb^{KO}$ [33] and $EMRE^{KO}$ [90]), a transgenic mouse expressing a heart-specific dominant-negative form of MCU ($DN\text{-}MCU^{TG}$), an inducible heart-specific MCU^{KO} ($ihMCU^{KO}$, [101,102]), and a cardiomyocyte-specific MCUb overexpressing mouse ($MCUb^{TG}$ [32,33]). Notably, these models exhibit minimal effects on fundamental cardiac functions based on standard parameters, such as ejection fraction or ventricular fractional shortening. Upon β-adrenergic stimulation, the rapid MCU-mediated increase in $[Ca^{2+}]_m$ was found to be essential in enhancing cardiac function and augmenting energy supply through the stimulation of mitochondrial metabolism in both $ihMCU^{KO}$ and $DN\text{-}MCU^{TG}$ mouse models [101–104]. Similarly, acute overexpression of MCUb, resulting in reduced MCU complex activity, led to impairment in the fight-or-flight response [32].

Regarding cardiomyocyte cell death during I/R injury, the acute deletion of MCU effectively prevented mitochondrial Ca^{2+} overload, mPT, and reduces the extent of myocardial damage [101,102]. Notably, during I/R injury, an increase in MCUb expression levels was reported, acting as a protective compensatory measure to reduce $[Ca^{2+}]_m$ and infarct area [32,33]. This was supported by increased cardiac remodelling and infarct area in $MCUb^{KO}$ mouse [33]. Coherently, prolonged MCUb overexpression in mouse hearts resulted in a reduced infarct size after I/R injury [32,33].

Surprisingly, in the total MCU^{KO} mouse [34], $EMRE^{KO}$ [90] and the $DN\text{-}Mcu^{TG}$ mouse [103], where rapid mitochondrial Ca^{2+} uptake is abolished, no significant differences in cardiomyocyte susceptibility to cell death during I/R injury were observed. Additionally, MCU^{KO} mouse did not exhibit sensitivity to the mPT inhibitor cyclosporine A (CsA) in protecting cardiomyocytes from damage following I/R injury [34]. A recent study by Parks et al. offered a plausible mechanism to reconcile the lack of sensitivity to CsA in the MCU^{KO} mouse. The researchers demonstrated that cardiac mitochondria in MCU^{KO} mice exhibit lower Ca^{2+} retention capacities when Ca^{2+} is driven into the mitochondrial matrix using the Ca^{2+} ionophore ETH-129. This phenomenon is attributed to higher levels of phosphorylated CypD, rendering mitochondria more susceptible to mPT, even in the absence of rapid Ca^{2+} uptake by the MCU complex [105].

Despite conflicting evidence has emerged regarding the beat-to-beat coupling between increases in $[Ca^{2+}]_m$ and $[Ca^{2+}]_{cyt}$ [96], mouse cardiomyocyte mitochondria appear to integrate the frequency of cytoplasmic Ca^{2+} signals as changes in steady-state $[Ca^{2+}]_m$ [26,111,112]. Mouse cardiac mitochondria show a lower maximal Ca^{2+} uptake capacity, a lower $[Ca^{2+}]$ threshold for activation, and a less steep

Table 1
HEART.

Model	Ca^{2+} phenotype	Basal heart functions	Tissue metabolism	Fight and flight response	I/R injury	Reference
Total MCU KO (germline) mouse	Abolished mitochondrial Ca^{2+}uptake and lower basal $[Ca^{2+}]_{mit}$	Impaired ability to perform exhausting physical activity but normal ejection.	Defect in Ca^{2+}-stimulated respiration	No effect	No effect, but impairment of the protective effect of CsA	[34, 105–107]
Heart-specific dominant-negative MCU mouse	Abolished mitochondrial Ca^{2+}uptake and lower basal $[Ca^{2+}]_{mit}$	Normal	Increased PDH phosphorylation levels	Impaired response	No effect	[103]
Heart-specific dominant-negative MCU mouse - pacemaker cells	Abolished mitochondrial Ca^{2+}uptake	Normal	Impaired ATP production upon increase heart frequency	Impaired chronotropic response	n.d.	[104]
Tamoxifen-inducible heart-specific MCU KO mouse	mitochondrial Ca^{2+}uptake, no changes in basal $[Ca^{2+}]_{mit}$	Normal	Impaired metabolism upon increase activity	Impaired response	Protection	[101,102]
Heart MICU1 overexpression mouse	Increased threshold and cooperativity for mitochondrial Ca^{2+} uptake.	Heart contractile impairment with decrease in ejection fraction and fractional shortening.	n.d.	n.d.	n.d.	[26]
Heart specific MICU1 silencing mouse -Intramyocardial injection	Mitochondrial Ca^{2+} overload	Normal	Altered mitochondrial morphology and suppressed mitochondrial function.	n.d.	Exacerbation (worsening infarct size, cardiac function, and myocardial apoptosis)	[108]
Inducible heart-specific MCU KO mouse	n.d.	Normal	Higher reliance on lipid oxidation under increased workload	No effect	n.d.	[109]
Acute MCUb overexpression mouse	Decrease mitochondrial Ca^{2+}uptake rate	Acute induction causes a transient impairment of contractile function (recover after 1 month)	Impaired maximal respiration, reserve capacity and increased PDH phosphorylation levels	Impaired response	Protection	[32]
Total MICU2 KO mouse	delayed cytosolic Ca^{2+} reuptake	Normal up to 16 months old, after left atrial enlargement.		n.d.	n.d.	[94]
Total EMRE KO mouse	Abolished mitochondrial Ca^{2+}uptake and lower basal $[Ca^{2+}]_{mit}$	Normal	No effect	No effect	No effect, but impairment of the protective effect of CsA	[90]
Inducible heart-specific EMRE KO mouse	Abolished mitochondrial Ca^{2+}uptake and lower basal $[Ca^{2+}]_{mit}$	Normal	Defect in Ca^{2+}-stimulated ATP production	Impaired response	Protection in the short-term	[110]
Total MCUb KO mouse	No changes in basal	Normal	Normal	n.d.	Exacerbation and increased cardiac-remodeling	[33]
Cronic MCUb overexpression mouse	Inhibited mitochondrial Ca^{2+}uptake	Normal	Normal	n.d.	Protection	[33]
Total MICU3 KO mouse	n.d.	Normal	Increased PDH phosphorylation levels	Protection against beta-adrenergic induced dysfunction	Protection	[46]

n.d.: not determined.

non-linear dependence on $[Ca^{2+}]$ compared to liver or muscle mitochondria [26]. These kinetic properties are influenced by the composition of the MCU complex, in particular the low ratio of MICU1:MCU content [26,27]. In this configuration, only an increase in frequency leads to a gradual increase in $[Ca^{2+}]_m$ [26]. Conversely, increasing the MICU1:MCU ratio, as achieved by MICU1 overexpression in the heart, imposes a higher threshold of activation and increases the cooperative activation of the channel, but causes contractile dysfunction [26]. Intriguingly, in biopsies of failing human hearts, the MICU1:MCU ratio is higher relative to samples from healthy patients and correlates with decreased ejection fraction [49]. Importantly, despite a lower MICU1:MCU ratio, silencing MICU1 in mouse cardiac mitochondria exacerbates I/R injury [108]. Furthermore, it has been shown that MICU1 levels are downregulated in the heart of diabetic *db/db* mice, which promotes intrinsic apoptosis in neonatal cardiomyocytes [50]. In addition, restoring MICU1 levels to normal improves cardiac function in *db/db* mice.

Overall, these studies suggest that $[Ca^{2+}]_m$ plays an essential role for cellular adaptation to increased energy demands during stress responses and for regulating substrate oxidation preferences. Under pathological conditions, the modulation of MCU channel activity protects against Ca^{2+}-induced mPT and cardiomyocyte cell death.

4.3. Skeletal muscle

Muscle contraction is vital for enabling body movement, maintaining posture and is essential for breathing process. To support muscle contraction, the production of ATP needs to be tightly regulated to meet the increased energy demands, which can rise by several orders of magnitude during intense physical activity [113,114]. In addition to their mechanical function, muscles also play crucial roles in the overall energy balance of the body. They act as storage sites for glucose during

post-prandial fasting and serve as sources of amino acids during periods of starvation [115].

In the context of muscle homeostasis, the role of mitochondrial Ca^{2+} signalling is well established (Table 2). Overexpression of MCU in muscle cells leads to muscle hypertrophy and provides protection against denervation-induced atrophy [116]. Conversely, muscle-specific knockdown ($skMCU^{KD}$) or muscle-specific MCU^{KO} ($skMCU^{KO}$) results in muscle atrophy and impaired strength and performance in mice [34,116–118]. Indeed, MCU-dependent mitochondrial Ca^{2+} uptake exerts a significant trophic effect on two major hypertrophic pathways in skeletal muscle, namely the PGC-1α4 and IGF1-Akt/PKB pathways [119,120]. In particularly, the PGC-1α4 pathway appears to be influenced by mitochondrial Ca^{2+} signalling, as demonstrated by conditions that increase mitochondrial Ca^{2+} uptake, such as the depletion of the cytosolic Ca^{2+} buffer parvalbumin, which affects muscle trophism and activates this pathway [121]. Moreover, $skMCU^{KO}$ mice exhibit a profound change in muscle composition, shifting from slow-twitch to fast-twitch muscle fibre types [117]. This shift is accompanied by a change in metabolic preference, characterized by increased glycolytic rate, reduced pyruvate utilization, and increased β-oxidation to supply mitochondrial oxidative catabolism [117,118]. The metabolic shift in $skMCU^{KO}$ mice is attributed to lower PDH activity, resulting from reduced steady-state $[Ca^{2+}]_{mit}$ [117]. A similar metabolic phenotype is observed in mice overexpressing the Pyruvate Dehydrogenase Kinase 4 (PDK4), which promotes PDH phosphorylation and inactivation. Interestingly, normal PDH activity and pyruvate oxidation can be restored in $skMCU^{KO}$ mice by overexpressing the Ca^{2+}-insensitive Pyruvate Dehydrogenase Phosphatase PDP2 [117]. Despite their higher reliance on fatty acid oxidation, $skMCU^{KO}$ myofibers exhibit elevated rates of glucose uptake and lactate production *in vivo* compared to controls, leading to increased blood lactate levels due to decreased pyruvate oxidation. This increased demand for glucose triggers a rewiring of whole-body metabolism in $skMCU^{KO}$ mice. This includes upregulated hepatic gluconeogenesis and glycogenolysis and increased lipolysis in visceral adipose tissue [117]. A similar metabolic phenotype was observed in a mouse model with tamoxifen-inducible MCU deletion in adulthood, suggesting that this outcome is not merely an adaptation response to chronic MCU deletion [117]. Altogether, these findings highlight the pivotal role of MCU in regulating muscle activity, trophism, and substrate oxidation preferences, with significant consequences for whole-body metabolism.

The significance of mitochondrial Ca^{2+} signalling in muscle physiology is further underscored by the higher Ca^{2+} transport currents exhibited by muscle mitochondria compared to other organs [39]. Myocyte mitochondria have a higher density of the MCU complex, facilitating mitochondrial Ca^{2+} uptake, particularly in comparison to cardiomyocytes and hepatocytes [26,27]. Furthermore, myocyte MCU complex contains MICU1.1, a tissue-specific splice variant of MICU1 with a significantly greater affinity for Ca^{2+}, promoting Ca^{2+}-induced MCU activation and ensuring efficient coupling of mitochondrial metabolism to meet ATP demands [43]. In skeletal muscle, MCU channel gating is primarily controlled by MICU1 homodimers rather than MICU1-MICU2 heterodimers [27]. It is worth noting that mutations in MICU1 have been associated with muscular and nervous system abnormalities in humans [93,124–126]. Consistent with these findings, mice with a muscle-specific deletion of MICU1 exhibit alteration in Ca^{2+} homeostasis, muscle weakness, atrophy, fatigue, and impaired sarcolemma repair abilities [122]. Indeed, the MCU channel has been implicated in plasma membrane repair through a mechanism involving Ca^{2+}-stimulated mitochondrial ROS production, initiating actin polymerization through the redox sensor RhoA [127]. Interestingly, patients with mutated MICU2 experience severe encephalopathy and cognitive impairment but do not show signs of myopathy [128], confirming the predominant role of MICU1 homodimer in regulating MCU in muscle cells or potential compensation by MICUs dimer shifts.

4.4. Liver

In addition to its exocrine and endocrine-like functions, the liver plays a pivotal role in whole-body energy homeostasis. It is involved in the uptake, storage, modification, and distribution of nutrients, thereby playing an essential part in the regulation of blood glucose levels and responses to stress. Many of these functions are finely regulated by Ca^{2+}, which are integral components of signalling cascades initiated by hormones like insulin, glucagon, and epinephrine [129].

Liver MCU complexes have a high MICU1:MCU ratio, which confers a high threshold and highly cooperative Ca^{2+} transport to mitochondria. This configuration allows rapid and efficient translocation of individual cytoplasmic Ca^{2+} spikes into mitochondria [26]. Notably, silencing MICU1 in mouse liver results in Ca^{2+} uptake patterns similar to those observed in cardiac mitochondria [26]. In mice, these channels are primarily regulated by MICU1:MICU2 heterodimers [27]. MICU1

Table 2
SKELETAL MUSCLE.

Model/Strain	Ca^{2+} phenotype	Muscle functions	Tissue metabolism	Reference
Skeletal muscle-specific MICU1 KO mouse	Loss of MCU complex threshold of activation and impaired maximal $[Ca^{2+}]m$	Muscle weakness, muscle atrophy and impaired fiber repairment	Muscle fatigue and enhanced anaerobic metabolism after exercise	[122]
Skeletal muscle-specific MCU KO mouse	Abolished mitochondrial Ca^{2+} uptake	Atrophy, impaired muscle force and exercise performance;	Slow-to-fast fibre type switch, impaired oxidative metabolism, increased fatty acid oxidation. Systemic metabolism alteration (liver and adipose tissue).	[117,118]
Inducible skeletal muscle-specific MCU KO mouse	n.d.	Atrophy, impaired exercise performance;	Slow-to-fast fibre type switch, impaired oxidative metabolism, increase fatty acid oxidation. Systemic metabolism alteration (lactate, FA and keto-bodies levels)	[117]
Skeletal muscle-MCU KD	Decreased mitochondrial Ca^{2+} uptake and basal $[Ca^{2+}]_{mit}$	Muscle fiber atrophy	Increased PDH phosphorylation	[116]
Skeletal muscle MCU overexpression	Increased mitochondrial Ca^{2+} uptake and basal $[Ca^{2+}]_{mit}$	Muscle fiber hypertrophy and protection from denervation-induced atrophy	Decrease PDH phosphorylation	[116]
Skeletal muscle-specific MCUb KO mouse	Impaired mitochondrial Ca^{2+} retention capacity	Normal	Increased pyruvate dehydrogenase activity, increased muscle malonyl coenzyme A (CoA), reduced fatty acid utilization, glucose intolerance, and increased adiposity.	[41]
Skeletal muscle-specific MCUb overexpression mouse	Increased mitochondrial Ca^{2+} retention capacity	n.d.	Increased fatty acid oxidation, decreased fat accumulation, and lower body weight.	[41]
Total MICU3 KO mouse	Decreased mitochondrial Ca^{2+} uptake	Normal	IIa-to-IIb fiber type switch, impaired exercise performance, muscle fatigue, increased PDH phosphorylation levels, reduced NADH production upon stimulation	[123]

n.d.: not determined.

silencing impairs cellular responses to hormones and makes cells more susceptible to Ca^{2+} overload ([22] and Table 3). However, under basal conditions *in vivo*, specific liver MICU1 KO mice do not show significant changes in liver morphology, histology or function. However, they fail to regenerate successfully after hepatectomy [91]. This regenerative failure has been attributed to mitochondrial Ca^{2+} overload, which leads to the induction of mPT and subsequent hepatocyte necrosis, resulting in persistent inflammation [91]. Liver regeneration is also impaired in MCU^{KO} mice [86] which dictates the balance between the Ca^{2+} elevation necessary for metabolic stimulation and the excessive levels that induce mPT [130].

The liver-specific deletion of MCU ($lMCU^{KO}$) provides valuable insights into the role of mitochondrial Ca^{2+} in hepatic metabolism [131]. Mitochondria from the liver of MCU^{KO} mice are unable of rapid Ca^{2+} uptake and exhibit lower basal $[Ca^{2+}]_{mit}$. Consequently, the ablation of MCU leads to impairments in fatty acid oxidation, resulting in the massive accumulation of lipids and reduced ketone body production, particularly under fasting conditions [131]. The underlying mechanisms of these alterations involve aberrant AMP-activated Protein Kinase (AMPK) activation. The impaired rapid Ca^{2+} uptake by mitochondria causes AMPK dephosphorylation. This dephosphorylation is mediated by the Ca^{2+}-dependent phosphatase PP4, even under conditions of a low ATP/AMP ratio, when AMPK should be active. Conversely, in a model of MCU hyperactivation due to an activating mutation of MCU (C96A) that results in higher basal and stimulated $[Ca^{2+}]_{mit}$, there is an increase in basal and maximal oxygen consumption rate. In this context, AMPK is highly phosphorylated, which leads to the activation of downstream pathways that reduced liver and plasma triglycerides, increased ketone bodies production, and enhanced lipid clearance during fasting conditions [131].

4.5. Brain

The brain metabolic demand accounts for approximately 20% of the total oxygen consumption in the body during resting or basal physiological states [132]. This sustained energy requirement is principally attributable to the maintenance of electrochemical ion gradients across the neuronal plasma membrane and the energetically demanding processes inherent in synaptic neurotransmission. Neurons in the brain require an efficient balance between ATP production through glycolytic pathways and mitochondrial oxidative phosphorylation to effectively meet the dynamic energy demands associated with varying neural activity [133]. In most cases, glucose serves as brain primary energy source, although during prolonged fasting or in ketogenic diets, the brain can also utilize ketone bodies for energy [134].

The large number of different cell types presents in the brain, with distinct metabolic profiles, excitable properties, channel composition, and mitochondrial heterogeneity [135–137], hinders a complete understanding of mitochondrial Ca^{2+} signalling in this organ (Table 4). Single-cell analysis has provided insight into the distribution of MCU components transcripts across various brain cell types. Notably, a robust enrichment of MCU, MICU1, and MICU2 is present in excitatory and inhibitory neurons, as well as in microglia and oligodendrocytes, and to a lesser extent in astrocytes [138,139]. Particularly noteworthy is the substantial enrichment of MICU3 in neurons and glia. MICU3 was originally identified as a neuronal specific MCU complex regulator, able to enhance mitochondrial Ca^{2+} signalling in this cell type [24]. Recently, it was demonstrated that MICU3 is essential in axonal mitochondria within neurons, where it lowers the threshold for MCU channel activation. This adaptation enables the rapid uptake of Ca^{2+} in response to small changes in local $[Ca^{2+}]_{cyt}$ and allows for metabolic flexibility, transitioning from glycolytic to oxidative metabolism during cellular activity [38]. Notably, within neurons, a cell type characterized by a highly specialized structure comprising dendrites, soma and long axon, there is a remarkable heterogeneity in the mitochondrial population. Recent reports underscore this heterogeneity by revealing significantly larger mitochondrial Ca^{2+} transients in the soma and apical dendrites compared to the proximal axon and basal dendrites [140]. This observation suggests the existence of distinct MCU complexes within different mitochondrial subpopulations, each potentially characterized by a specific repertoire of regulatory subunits capable of modulating the activation properties of the MCU channels.

Furthermore, disruptions in $[Ca^{2+}]_{cyt}$ homeostasis leading to subsequent mitochondrial Ca^{2+} overload, often culminating in mPT, have been identified as pivotal mechanisms underlying cellular injury during excitotoxicity, I/R in neurons, and prevalent pathological hallmarks in various neurodegenerative disorders [100,146]. Consequently, the investigation of the MCU complex in neurons holds significant relevance as a potential target for therapeutic intervention. For instance, pharmacological inhibition of the MCU complex with Ru360 [147] or acute KD of MCU [148] has been demonstrated to protect neurons against excitotoxicity in *in vitro* experiments. An interesting observation is that the activation of neurons, inducing sub-toxic elevations in cytoplasmic Ca^{2+} levels that mimic physiological activation, results in the down-regulation of MCU expression [136,148], possibly as protective mechanism to prevent mitochondrial Ca^{2+} overload. Indeed, the overexpression of MCU can have deleterious effects on neural function. MCU overexpression exacerbates the loss of mitochondrial membrane potential ($\Delta\Psi$) induced by NMDA and promotes neuron death, even under normal physiological conditions [144,148]. Indeed, the introduction of adenoviral particles carrying the MCU gene into the brain cortex elicits gliosis, microglial activation, and neural loss [144].

Similarly to the heart, global MCU^{KO} mice did not manifest protection against I/R damage in the brain [141,142]. Moreover, the elimination of MCU resulted in the loss of the protective effects induced by hypoxic preconditioning in the brain [141]. To further investigate these findings, MCU deletion was induced in adult animals using a tamoxifen-driven Cre-recombinase system, specifically targeting Thy1-positive neurons [143]. In these adult-induced neuronal MCU^{KO} mice, reductions in sensorimotor deficits, brain damage, infarct volume,

Table 3
LIVER.

Model/Strain	Ca^{2+} phenotype	Liver functions	Tissue metabolism	Reference
Liver specific MICU1 KD	Loss of threshold and cooperativity activation of MCU	Normal under basal conditions. Liver is unable to regenerate after hepatectomy (decrease hepatocytes proliferation, massive necrosis and persistent inflammation).	Defect in Ca^{2+}-stimulated ATP production	[22,91]
Total MICU2 KO mouse	Increased $[Ca^{2+}]_{mit}$ accumulation slope upon low $[Ca^{2+}]$ stimulation	n.d.	n.d.	[94]
Liver specific MCU KO mouse	Abolished mitochondrial Ca^{2+} uptake	accumulation of lipids, and lower ketone body production during fasting.	Compromised fatty acid oxidation and decreased ATP level	[131]
MCU hyperactivation (MCU^{C96A} KI) mouse	Increased mitochondrial Ca^{2+} uptake and basal $[Ca^{2+}]_{mit}$	Reduced liver and plasma TAG; increased ketone bodies production and lipid clearance during fasting.	Increased oxidative metabolism	[131]

n.d.: not determined.

Table 4
BRAIN.

Model/Strain	Ca^{2+} phenotype	Brain functions	Metabolism	Neuronal cell injury	Reference
Total MCU KO mouse	Abolished mitochondrial Ca^{2+} uptake	Normal	Hyperphosphorylation of PDH, and impairment of oxidative metabolism during hypoxia.	No protection against Hypoxic-Ischemic injury and loss of hypoxic preconditioning protection.	[141, 142]
Inducible neuronal-specific MCU KO mouse	n.d.	Normal	No effect	Protection against Hypoxic-Ischemic injury.	[143]
MCU overexpression in the brain cortex	Increased mitochondrial Ca^{2+} uptake and basal $[Ca^{2+}]_{mit}$ (*in vitro*)	Neuronal degeneration (*in vivo*)	n.d.	Exacerbation of excitotoxic cell death (*in vitro*)	[144]
$MCU^{+/-}$ mouse	n.d.	n.d.	n.d.	Protection against Hypoxic-Ischemic injury (Reduced mitochondrial Ca^{2+} overload, infarct volume, neuron apoptosis).	[142]
Neuron-specific MICU1 KO mouse	Increased basal $[Ca^{2+}]_{mit}$ and mitochondria are prone to Ca^{2+} overload (*in vitro*)	Progressive cognitive and motor impairment	n.d.	Exacerbation of the excitotoxic cell death (*in vitro*)	[145]

n.d.: not determined.

and mitochondrial injuries were observed following I/R. Likewise, MCU heterozygous mice ($MCU^{+/-}$), characterized by a 40% reduction in MCU protein levels, exhibited reduced mitochondrial Ca^{2+} overload, reduced infarct volume, diminished neuron apoptosis, preserved neuron ultrastructure, and ameliorated neurological deficits post-cerebral infarction [142].

In recent years, an increasing number of patients exhibiting neuromuscular and cognitive impairments have been identified with loss-of-function mutations in MICU1 [93,124–126]. These studies strongly imply a direct association between patient symptoms and altered mitochondrial Ca^{2+} signalling. Consequently, a neuronal-specific MICU1 KO mouse model ($nMICU1^{KO}$) was developed to elucidate the regulatory role of the MCU complex in neuronal biology and neurological impairments [145]. The $nMICU1^{KO}$ mouse exhibits a progressive, abnormal motor and cognitive phenotype, accompanied by the degeneration of motor neurons in the spinal cord and cortex. Notably, these manifestations are concomitant with a lower Ca^{2+} activation threshold and maximal Ca^{2+} uptake capacity in isolated cortical mitochondria.

The collective results from both *in vitro* and *in vivo* models strongly support the crucial role of mitochondrial Ca^{2+} signalling in neurons, underscoring the critical importance of finely tuning $[Ca^{2+}]_{mit}$ to sustain neuronal activity and prevent deleterious Ca^{2+} overload. The observed discrepancies between constitutive and inducible MCU^{KO} models further emphasize that chronic MCU deletion may elicit diverse compensatory mechanisms.

4.6. Pancreatic β cells

Pancreatic β cells play a pivotal role in regulating glucose homeostasis as they serve as the primary source of the hormone insulin. The release of insulin is governed by a process called glucose-induced insulin secretion (GSIS): when glucose levels increase in pancreatic β cells, it stimulates aerobic metabolism, leading to increased ATP production. This rise in ATP levels results in the closure of ATP-sensitive K^+ channels, membrane depolarization, and subsequent influx of Ca^{2+} via voltage-gated Ca^{2+} channels, ultimately triggering insulin release [149]. Impairments in β-cell glucose sensitivity and reductions in β-cell mass are fundamental to the development of insulin-resistant diabetes mellitus [150]. Given the role of mitochondrial Ca^{2+} in regulating aerobic metabolism, it is unsurprising that $[Ca^{2+}]_{mit}$ plays a role in β cell functions [151]. The involvement of MCU channel components has been investigated in various β cell derived cell lines such as INS-1 cells and rat islets [152,153]. Specific deletion of MCU in β cells impairs glucose stimulated mitochondrial Ca^{2+} accumulation, ATP production, and insulin secretion *ex vivo*. *In vivo* studies have shown that the deletion of MCU in β cells leads to significantly higher blood glucose levels following glucose challenge due to impaired insulin release [154]. Additionally, $MICU2^{KO}$ mice exhibit impaired GSIS, potentially linked to a disruption in mitochondrial Ca^{2+} uptake [155]. Overall, mitochondrial Ca^{2+} signalling plays a crucial role in the insulin secretion process by sustaining ATP production, which signals for the release of insulin.

5. Conclusions and perspectives

This review aims to highlight the critical role of mitochondrial Ca^{2+} signalling in various physiological contexts, drawing from recent studies conducted in different cell types and animal models. These studies have significantly contributed to our understanding of the MCU channel intricate system, revealing how the molecular composition and the regulatory mechanisms can vary based on tissue specificity. The diverse phenotypes observed in various animal models highlight the importance of integrating findings to gain a comprehensive understanding of mitochondrial Ca^{2+} signalling across organisms. Given the critical role of mitochondrial Ca^{2+} dysregulation in numerous pathologies, understanding the efficient control and maintenance of the MCU complex becomes imperative. To achieve this, standardization of experimental approaches and techniques is crucial to reconcile conflicting results. For instance, it has emerged that studies employing mitoplast patch clamp recordings must be meticulously conducted to ensure comprehensive analysis of the entire MCU complex, ensuring accuracy and reliability [67,75,76]. Continued advancements in Ca^{2+} probes, microscopy techniques offering higher resolution, and *in vivo* Ca^{2+} measurements are essential for advancing the field and validating results obtained in cell lines and isolated mitochondria. These developments will enable researchers to delve deeper into the function and regulation mechanisms of mitochondrial Ca^{2+} signalling. While significant progress has been made in identifying various molecules that modulate MCU channel activity [156–159], their clinical implementation remains distant due to their non-specific effects. The challenge lies in their low specificity and broad action, affecting mitochondria across various tissues indiscriminately. The discovery of new molecules and new therapeutic strategies that act at different levels of the MCU channel regulatory layers, and an understanding of how different tissues uniquely modulate MCU channel activity, may pave the way for more tailored pharmacological interventions. This targeted approach holds great promise for advancing the clinical translation of MCU channel modulators and ultimately improving the treatment outcomes in several pathologies linked to mitochondrial Ca^{2+} dysregulation.

Funding resources

This research was supported with funding from the Italian Ministry

of University and Research (PRIN 20207P85MH to AR) and the European Union (Next-Generation EU CN00000041). DVR was supported by European Union HORIZON-MSCA-2021-PF 101065790.

CRediT authorship contribution statement

Denis Vecellio Reane: Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Julian D.C. Serna:** Writing – original draft. **Anna Raffaello:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization.

Declaration of competing interest

Authors declare no conflict of interests.

Data availability

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

Acknowledgements

All figures were created using BioRender.com.

References

[1] F.D. Vasington, J.V Murphy, Ca ion uptake by rat kidney mitochondria and its dependence on respiration and phosphorylation, J. Biol. Chem. 237 (1962) 2670–2677. http://www.ncbi.nlm.nih.gov/pubmed/13925019. accessed January 16, 2016.

[2] H.F. DeLuca, G.W. Engstrom, CALCIUM UPTAKE BY RAT KIDNEY MITOCHONDRIA, Proc. Natl. Acad. Sci. 47 (1961) 1744–1750, https://doi.org/10.1073/pnas.47.11.1744.

[3] R.M. Denton, J.G. McCormack, N.J. Edgell, Role of calcium ions in the regulation of intramitochondrial metabolism. Effects of Na+, Mg2+ and ruthenium red on the Ca2+-stimulated oxidation of oxoglutarate and on pyruvate dehydrogenase activity in intact rat heart mitochondria, Biochem. J. 190 (1980) 107–117, https://doi.org/10.1042/bj1900107.

[4] A. Rasola, P. Bernardi, Mitochondrial permeability transition in Ca(2+)-dependent apoptosis and necrosis, Cell Calcium 50 (2011) 222–233, https://doi.org/10.1016/j.ceca.2011.04.007.

[5] D. D'Angelo, D. Vecellio Reane, A. Raffaello, Neither too much nor too little: mitochondrial calcium concentration as a balance between physiological and pathological conditions, Front. Mol. Biosci. 10 (2023) 1–8, https://doi.org/10.3389/fmolb.2023.1336416.

[6] J. Huo, J.D. Molkentin, MCU genetically altered mice suggest how mitochondrial Ca2+ regulates metabolism, Trends Endocrinol. Metab. (2024) 1–11, https://doi.org/10.1016/j.tem.2024.04.005.

[7] S.H. Lee, H.E. Duron, D. Chaudhuri, Beyond the TCA cycle: new insights into mitochondrial calcium regulation of oxidative phosphorylation, Biochem. Soc. Trans. 51 (2023) 1661–1673, https://doi.org/10.1042/BST20230012.

[8] 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 Ca(2+) uptake, Nature 467 (2010) 291–296, https://doi.org/10.1038/nature09358.

[9] D. De Stefani, A. Raffaello, E. Teardo, I. Szabò, 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.

[10] J.M. Baughman, F. Perocchi, H.S. Girgis, M. Plovanich, C.A. Belcher-Timme, Y. Sancak, X.R.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.

[11] A. De Mario, D. D'Angelo, G. Zanotti, A. Raffaello, C. Mammucari, The mitochondrial calcium uniporter complex–A play in five acts, Cell Calcium 112 (2023) 102720, https://doi.org/10.1016/J.CECA.2023.102720.

[12] S. Feno, R. Rizzuto, A. Raffaello, D. Vecellio Reane, The molecular complexity of the Mitochondrial Calcium Uniporter, Cell Calcium 93 (2021) 102322, https://doi.org/10.1016/J.CECA.2020.102322.

[13] B.R. Alevriadou, A. Patel, M. Noble, S. Ghosh, V.M. Gohil, P.B. Stathopulos, M. Madesh, Molecular nature and physiological role of the mitochondrial calcium uniporter channel, Am. J. Physiol. - Cell Physiol. 320 (2021) C465–C482, https://doi.org/10.1152/ajpcell.00502.2020.

[14] 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) 1379–1382, https://doi.org/10.1126/science.1242993.

[15] 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.

[16] 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.

[17] 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.

[18] E. Kovács-Bogdán, Y. Sancak, K.J. Kamer, M. Plovanich, A. Jambhekar, R. J. Huber, M.A. Myre, M.D. Blower, V.K. Mootha, E. Kovacs-Bogdan, 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.

[19] 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.

[20] D.M. Colussi, P.B. Stathopulos, From passage to inhibition: Uncovering the structural and physiological inhibitory mechanisms of MCUb in mitochondrial calcium regulation, FASEB J 37 (2023) e22678, https://doi.org/10.1096/FJ.202201080R.

[21] 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.

[22] G. Csordás, T. Golenár, E.L. Seifert, K.J. Kamer, Y. Sancak, F. Perocchi, C. Moffat, D. Weaver, S. de la Fuente Perez, R. Bogorad, V. Koteliansky, J. Adijanto, V. K. Mootha, G. Hajnóczky, S. de la F. Perez, R. Bogorad, V. Koteliansky, J. Adijanto, V.K. Mootha, G. Hajnóczky, MICU1 controls both the threshold and cooperative activation of the mitochondrial Ca^{2+} uniporter, Cell Metab 17 (2013) 976–987, https://doi.org/10.1016/j.cmet.2013.04.020.

[23] M. Patron, V. Checchetto, A. Raffaello, E. Teardo, D. Vecellio Reane, M. Mantoan, V. Granatiero, I. Szabò, D. De Stefani, 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.

[24] M. Patron, V. Granatiero, J. Espino, R. Rizzuto, D. De Stefani, MICU3 is a tissue-specific enhancer of mitochondrial calcium uptake, Cell Death Differ 26 (2019) 179–195, https://doi.org/10.1038/s41418-018-0113-8.

[25] K.J. Kamer, V.K. Mootha, MICU1 and MICU2 play nonredundant roles in the regulation of the mitochondrial calcium uniporter, EMBO Rep 15 (2014) 299–307, https://doi.org/10.1002/embr.201337946.

[26] M. Paillard, G. Csordás, G. Szanda, T. Golenár, V. Debattisti, A. Bartok, N. Wang, C. Moffat, E.L. Seifert, A. Spät, G. Hajnóczky, G. Rgy Csordá, G. Szanda, T. Unde Golená, V. Debattisti, A. Bartok, N. Wang, C. Moffat, E.L. Seifert, A.S. Spä, G. Rgy, H. Czky, 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 Ca 2+ 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.

[27] C.W. Tsai, M.X. Rodriguez, A.M. Van Keuren, C.B. Phillips, H.M. Shushunov, J. E. Lee, A.M. Garcia, A.V. Ambardekar, J.C. Cleveland, J.A. Reisz, C. Proenza, K. C. Chatfield, M.F. Tsai, Mechanisms and significance of tissue-specific MICU regulation of the mitochondrial calcium uniporter complex, Mol. Cell 82 (2022) 3661–3676, e8, http://www.cell.com/article/S1097276522008954/fulltext. accessed January 22, 2023.

[28] T.E. Gunter, D.R. Pfeiffer, Mechanisms by which mitochondria transport calcium, Am. J. Physiol. - Cell Physiol. 258 (1990), https://doi.org/10.1152/AJPCELL.1990.258.5.C755.

[29] H. Vais, R. Payne, U. Paudel, C. Li, J.K. Foskett, Coupled transmembrane mechanisms control MCU-mediated mitochondrial Ca2+uptake, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 21731–21739, https://doi.org/10.1073/PNAS.2005976117/SUPPL_FILE/PNAS.2005976117.SAPP.PDF.

[30] H. Vais, K. Mallilankaraman, D.O.D. Mak, H. Hoff, R. Payne, J.E. Tanis, J. K. Foskett, EMRE Is a Matrix Ca2+ Sensor that Governs Gatekeeping of the Mitochondrial Ca2+ Uniporter, Cell Rep 14 (2016) 403–410, https://doi.org/10.1016/J.CELREP.2015.12.054.

[31] S.K. Lee, S. Shanmughapriya, M.C.Y. Mok, Z. Dong, D. Tomar, E. Carvalho, S. Rajan, M.S. Junop, M. Madesh, P.B. Stathopulos, Structural Insights into Mitochondrial Calcium Uniporter Regulation by Divalent Cations, Cell Chem. Biol. 23 (2016) 1157–1169, https://doi.org/10.1016/j.chembiol.2016.07.012.

[32] J.P. Lambert, T.S. Luongo, D. Tomar, P. Jadiya, E. Gao, X. Zhang, A.M. Lucchese, D.W. Kolmetzky, N.S. Shah, J.W. Elrod, MCUB Regulates the Molecular Composition of the Mitochondrial Calcium Uniporter Channel to Limit Mitochondrial Calcium Overload during Stress, Circulation 140 (2019) 1720–1733, https://doi.org/10.1161/CIRCULATIONAHA.118.037968.

[33] J. Huo, S. Lu, J.Q. Kwong, M.J. Bround, K.M. Grimes, M.A. Sargent, M.E. Brown, M.E. Davis, D.M. Bers, J.D. Molkentin, MCUb Induction Protects the Heart From Postischemic Remodeling, Circ. Res. 127 (2020) 379–390, https://doi.org/10.1161/CIRCRESAHA.119.316369.

[34] X. Pan, J. Liu, T. Nguyen, C. Liu, J. Sun, Y. Teng, M.M. Fergusson, I.I. Rovira, M. Allen, D.A. Springer, A.M. Aponte, M. Gucek, R.S. Balaban, E. Murphy, T. Finkel, The physiological role of mitochondrial calcium revealed by mice

lacking the mitochondrial calcium uniporter, Nat. Cell Biol. 15 (2013) 1464–1472, https://doi.org/10.1038/ncb2868.
[35] 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) e55785, https://doi.org/10.1371/journal.pone.0055785.
[36] K. Mallilankaraman, P. Doonan, C. Cárdenas, C. Harish, M. Muller, R. Miller, N. E. Hoffman, J. Molgó, M.J. Birnbaum, B.S. Rothberg, D.-O.D. Mak, J.K. Foskett, M. Madesh, 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.
[37] K.J. Kamer, Z. Grabarek, V.K. Mootha, High-affinity cooperative Ca 2+ binding by MICU1–MICU2 serves as an on–off switch for the uniporter, EMBO Rep 18 (2017) e201643748, https://doi.org/10.15252/embr.201643748.
[38] 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.
[39] 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.
[40] S. Feno, F. Munari, D.V. 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 (2021) 3838, https://doi.org/10.1126/SCISIGNAL.ABF3838/SUPPL_FILE/SCISIGNAL.ABF3838_SM.PDF.
[41] J. Huo, V. Prasad, K.M. Grimes, D. Vanhoutte, N.S. Blair, S.-C. Lin, M.J. Bround, D.M. Bers, J.D. Molkentin, MCUb is an inducible regulator of calcium-dependent mitochondrial metabolism and substrate utilization in muscle, Cell Rep 42 (2023) 113465, https://doi.org/10.1016/j.celrep.2023.113465.
[42] F. Cividini, B.T. Scott, J. Suarez, D.E. Casteel, S. Heinz, A. Dai, T. Diemer, J. A. Suarez, C.W. Benner, M. Ghassemian, W.H. Dillmann, Ncor2/PPARα-Dependent Upregulation of MCUb in the Type 2 Diabetic Heart Impacts Cardiac Metabolic Flexibility and Function, Diabetes 70 (2021) 665–679, https://doi.org/10.2337/DB20-0779.
[43] 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), https://doi.org/10.1016/j.molcel.2016.10.001.
[44] D. Vecellio Reane, C. Cerqua, S. Sacconi, L. Salviati, E. Trevisson, A. Raffaello, The Splicing of the Mitochondrial Calcium Uniporter Genuine Activator MICU1 Is Driven by RBFOX2 Splicing Factor during Myogenic Differentiation, Int. J. Mol. Sci. 23 (2022) 2517, https://doi.org/10.3390/ijms23052517.
[45] Y.F. Yang, W. Yang, Z.Y. Liao, Y.X. Wu, Z. Fan, A. Guo, J. Yu, Q.N. Chen, J.H. Wu, J. Zhou, Q. Xiao, MICU3 regulates mitochondrial Ca2+-dependent antioxidant response in skeletal muscle aging, Cell Death Dis. 12 (2021) 1–13, https://doi.org/10.1038/s41419-021-04400-5, 2021 1212.
[46] 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.
[47] S.L. Menezes-Filho, I. Amigo, F.M. Prado, N.C. Ferreira, M.K. Koike, I.F.D. Pinto, S. Miyamoto, E.F.S. Montero, M.H.G. Medeiros, A.J. Kowaltowski, Caloric restriction protects livers from ischemia/reperfusion damage by preventing Ca2+-induced mitochondrial permeability transition, Free Radic, Biol. Med. 110 (2017) 219–227, https://doi.org/10.1016/J.FREERADBIOMED.2017.06.013.
[48] J.D.C. Serna, A.G. Amaral, C.C. Caldeira da Silva, A.C. Munhoz, E.A. Vilas-Boas, S. L. Menezes-Filho, A.J. Kowaltowski, Regulation of kidney mitochondrial function by caloric restriction, Am. J. Physiol. - Ren. Physiol. 323 (2022) F92–F106, https://doi.org/10.1152/AJPRENAL.00461.2021/ASSET/IMAGES/LARGE/AJPRENAL.00461.2021_F007.JPEG.
[49] M. Paillard, K.T. Huang, D. Weaver, J.P. Lambert, J.W. Elrod, G. Hajnóczky, Altered composition of the mitochondrial Ca2+uniporter in the failing human heart, Cell Calcium 105 (2022) 102618, https://doi.org/10.1016/J.CECA.2022.102618.
[50] L. Ji, F. Liu, Z. Jing, Q. Huang, Y. Zhao, H. Cao, J. Li, C. Yin, J. Xing, F. Li, MICU1 Alleviates Diabetic Cardiomyopathy Through Mitochondrial Ca(2+)-Dependent Antioxidant Response, Diabetes 66 (2017) 1586–1600, https://doi.org/10.2337/db16-1237.
[51] I.Y. Kuo, A.L. Brill, F.O. Lemos, J.Y. Jiang, J.L. Falcone, E.P. Kimmerling, Y. Cai, K. Dong, D.L. Kaplan, D.P. Wallace, A.M. Hofer, B.E. Ehrlich, Polycystin 2 regulates mitochondrial Ca2+ signaling, bioenergetics, and dynamics through mitofusin 2, Sci. Signal. 12 (2019), https://doi.org/10.1126/SCISIGNAL.AAT7397/SUPPL_FILE/AAT7397_SM.PDF.
[52] M. a. Joiner, O.M. Koval, J. Li, B.J. 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.
[53] 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.
[54] 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.
[55] 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 Ca 2+ Uptake Through Pyk2-Dependent Tyrosine Phosphorylation of the Mitochondrial Ca 2+ Uniporter, Antioxid. Redox Signal. 21 (2014) 863–879, https://doi.org/10.1089/ars.2013.5394.
[56] 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.
[57] 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, 2019 214.
[58] 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 MICU 1 regulates mitochondrial Ca 2+ levels and tumor growth, EMBO J 38 (2019) 1–20, https://doi.org/10.15252/embj.201899435.
[59] 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.
[60] E. Balderas, D.R. Eberhardt, S. Lee, J.M. Pleinis, S. Sommakia, A.M. Balynas, X. Yin, M.C. Parker, C.T. Maguire, S. Cho, M.W. Szulik, A. Bakhtina, R.D. Bia, M. W. Friederich, T.M. Locke, J.L.K. Van Hove, S.G. Drakos, Y. Sancak, M. Tristani-Firouzi, S. Franklin, A.R. Rodan, D. Chaudhuri, Mitochondrial calcium uniporter stabilization preserves energetic homeostasis during Complex I impairment, Nat. Commun. 13 (2022) 1–17, https://doi.org/10.1038/s41467-022-30236-4, 2022 131.
[61] 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.
[62] M. Trenker, R. Malli, I. Fertschai, S. Levak-Frank, W.F. Graier, Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca2+ uniport, Nat. Cell Biol. 9 (2007) 445–452, https://doi.org/10.1038/ncb1556.
[63] A.I. Bondarenko, W. Parichatikanond, C.T. Madreiter, R. Rost, M. Waldeck-Weiermair, R. Malli, W.F. Graier, UCP2 modulates single-channel properties of a MCU-dependent Ca(2+) inward current in mitochondria, Pflugers Arch 467 (2015) 2509–2518, https://doi.org/10.1007/s00424-015-1727-z.
[64] 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.
[65] C.-W.W. Tsai, Y. Wu, P.-C.C. Pao, C.B. Phillips, C. Williams, C. Miller, M. Ranaghan, M.-F.F. Tsai, Proteolytic control of the mitochondrial calcium uniporter complex, Proc. Natl. Acad. Sci. 114 (2017) 4388–4393, https://doi.org/10.1073/PNAS.1702938114.
[66] R. Payne, C. Li, J.K. Foskett, Variable Assembly of EMRE and MCU Creates Functional Channels with Distinct Gatekeeping Profiles, IScience 23 (2020) 101037, https://doi.org/10.1016/J.ISCI.2020.101037.
[67] V. Garg, J. Suzuki, I. Paranjpe, T. Unsulangi, L. Boyman, L.S. Milescu, W. J. Lederer, Y. Kirichok, W.Jonathan Lederer, Y. Kirichok, W.J. Lederer, Y. Kirichok, The mechanism of MICU-dependent gating of the mitochondrial Ca2+uniporter, Elife 10 (2021), https://doi.org/10.7554/eLife.69312.
[68] K. Mallilankaraman, C. Cárdenas, P.J. Doonan, H.C. Chandramoorthy, K. M. Irrinki, T. Golenár, G. Csordás, P. Madireddi, J. Yang, M. Müller, R. Miller, J. E. Kolesar, J. Molgó, B. Kaufman, G. Hajnóczky, J.K. Foskett, M. Madesh, MCUR1 is an essential component of mitochondrial Ca2+ uptake that regulates cellular metabolism, Nat. Cell Biol. 14 (2012) 1336–1343, https://doi.org/10.1038/ncb2622.
[69] 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.
[70] 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.
[71] 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.
[72] B. Gottschalk, C. Klec, G. Leitinger, E. Bernhart, R. Rost, H. Bischof, C. T. Madreiter-Sokolowski, S. Radulović, E. Eroglu, W. Sattler, M. Waldeck-Weiermair, R. Malli, W.F. Graier, MICU1 controls cristae junction and spatially anchors mitochondrial Ca2+ uniporter complex, Nat. Commun. 10 (2019) 1–17, https://doi.org/10.1038/s41467-019-11692-x, 2019 101.
[73] B. Gottschalk, Z. Koshenov, M. Waldeck-Weiermair, S. Radulović, F.E. Oflaz, M. Hirtl, O.A. Bachkoenig, G. Leitinger, R. Malli, W.F. Graier, MICU1 controls spatial membrane potential gradients and guides Ca2+ fluxes within mitochondrial substructures, Commun. Biol. 5 (2022), https://doi.org/10.1038/s42003-022-03606-3.
[74] D. Tomar, M. Thomas, J.F. Garbincius, D.W. Kolmetzky, O. Salik, P. Jadiya, S. K. Joseph, A.C. Carpenter, G. Hajnóczky, J.W. Elrod, MICU1 regulates mitochondrial cristae structure and function independently of the mitochondrial Ca2+ uniporter channel, Sci. Signal. 16 (2023) eabi8948, https://doi.org/10.1126/SCISIGNAL.ABI8948/SUPPL_FILE/SCISIGNAL.ABI8948_MDAR_REPRODUCIBILITY_CHECKLIST.PDF.
[75] C.-W. Tsai, T.-Y. Liu, F.-Y. Chao, Y.-C. Tu, M.X. Rodriguez, A.M. Van Keuren, Z. Ma, J. Bankston, M.-F. Tsai, Evidence supporting the MICU1 occlusion mechanism and against the potentiation model in the mitochondrial calcium uniporter complex, Proc. Natl. Acad. Sci. 120 (2023) 2017, https://doi.org/10.1073/pnas.2217665120.
[76] M. Rodríguez-Prados, E. Berezhnaya, M.T. Castromonte, S.L. Menezes-Filho, M. Paillard, G. Hajnóczky, MICU1 occludes the mitochondrial calcium uniporter in divalent-free conditions, Proc. Natl. Acad. Sci. U. S. A. 120 (2023) e2218999120, https://doi.org/10.1073/PNAS.2218999120/SUPPL_FILE/PNAS.2218999120.SAPP.PDF.
[77] S.D. Kaye, S. Goyani, D. Tomar, MICU1's calcium sensing beyond mitochondrial calcium uptake, Biochim. Biophys. Acta - Mol. Cell Res. 1871 (2024) 119714, https://doi.org/10.1016/j.bbamcr.2024.119714.
[78] M. Waldeck-Weiermair, R. Malli, W. Parichatikanond, B. Gottschalk, C. T. Madreiter-Sokolowski, C. Klec, R. Rost, W.F. Graier, Rearrangement of MICU1 multimers for activation of MCU is solely controlled by cytosolic Ca(2.), Sci. Rep. 5 (2015) 15602, https://doi.org/10.1038/srep15602.
[79] C. Petrungaro, K.M. Zimmermann, V. Küttner, M. Fischer, J. Dengjel, I. Bogeski, J. Riemer, The Ca(2+)-Dependent Release of the Mia40-Induced MICU1-MICU2 Dimer from MCU Regulates Mitochondrial Ca(2+) Uptake, Cell Metab 22 (2015) 721–733, https://doi.org/10.1016/j.cmet.2015.08.019.
[80] A. Matteucci, M. Patron, D. Vecellio Reane, S. Gastaldello, S. Amoroso, R. Rizzuto, M. Brini, A. Raffaello, T. Calì, Parkin-dependent regulation of the MCU complex component MICU1, Sci. Reports 8 (2018) 1–13, https://doi.org/10.1038/s41598-018-32551-7, 2018 81.
[81] A.D. Langenbacher, H. Shimizu, W. Hsu, Y. Zhao, A. Borges, C. Koehler, J. N. Chen, Mitochondrial Calcium Uniporter Deficiency in Zebrafish Causes Cardiomyopathy With Arrhythmia, Front. Physiol. 11 (2020) 617492, https://doi.org/10.3389/FPHYS.2020.617492/BIBTEX.
[82] C.M. Bisbach, R.A. Hutto, D. Poria, W.M. Cleghorn, F. Abbas, F. Vinberg, V. J. Kefalov, J.B. Hurley, S.E. Brockerhoff, Mitochondrial Calcium Uniporter (MCU) deficiency reveals an alternate path for Ca2+ uptake in photoreceptor mitochondria, Sci. Reports 10 (2020) 1–19, https://doi.org/10.1038/s41598-020-72708-x, 2020 101.
[83] S.K. Soman, M. Bazała, M. Keatinge, O. Bandmann, J. Kuznicki, Restriction of mitochondrial calcium overload by mcu inactivation renders a neuroprotective effect in zebrafish models of Parkinson's disease, Biol. Open 8 (2019), https://doi.org/10.1242/BIO.044347/266038/AM/RESTRICTION-OF-MITOCHONDRIAL-CALCIUM-OVERLOAD-BY.
[84] S. Xu, A.D. Chisholm, C. elegans Epidermal Wounding Induces a Mitochondrial ROS Burst that Promotes Wound Repair, Dev. Cell 31 (2014) 48–60, https://doi.org/10.1016/J.DEVCEL.2014.08.002.
[85] R. Tufi, T.P. Gleeson, S. von Stockum, V.L. Hewitt, J.J. Lee, A. Terriente-Felix, A. Sanchez-Martinez, E. Ziviani, A.J. Whitworth, Comprehensive Genetic Characterization of Mitochondrial Ca2+ Uniporter Components Reveals Their Different Physiological Requirements In Vivo, Cell Rep 27 (2019) 1541–1550, https://doi.org/10.1016/J.CELREP.2019.04.033, e5.
[86] O.M. Koval, E.K. Nguyen, V. Santhana, T.P. Fidler, S.C. Sebag, T.P. Rasmussen, D. J. Mittauer, S. Strack, P.C. Goswami, E.D. Abel, I.M. Grumbach, Loss of MCU prevents mitochondrial fusion in G 1 -S phase and blocks cell cycle progression and proliferation, Sci. Signal. 12 (2019) 1439, https://doi.org/10.1126/SCISIGNAL.AAV1439/SUPPL_FILE/AAV1439_SM.PDF.
[87] C. Cárdenas, M. Müller, A. McNeal, A. Lovy, F. Jaña, G. Bustos, F. Urra, N. Smith, J. Molgó, J.A. Diehl, T.W. Ridky, J.K. Foskett, Selective Vulnerability of Cancer Cells by Inhibition of Ca2+ Transfer from Endoplasmic Reticulum to Mitochondria, Cell Rep 14 (2016) 2313–2324, https://doi.org/10.1016/j.celrep.2016.02.030.
[88] J. Humeau, J.M. Bravo-San Pedro, I. Vitale, L. Nuñez, C. Villalobos, G. Kroemer, L. Senovilla, Calcium signaling and cell cycle: Progression or death, Cell Calcium 70 (2018) 3–15, https://doi.org/10.1016/J.CECA.2017.07.006.
[89] A. Weiser, A. Hermant, F. Bermont, F. Sizzano, S. Karaz, P. Alvarez-Illera, J. Santo-Domingo, V. Sorrentino, J.N. Feige, U. De Marchi, The mitochondrial calcium uniporter (MCU) activates mitochondrial respiration and enhances mobility by regulating mitochondrial redox state, Redox Biol 64 (2023) 102759, https://doi.org/10.1016/J.REDOX.2023.102759.
[90] J.C.J. Liu, N.C. Syder, N.S. Ghorashi, T.B. Willingham, R.J. Parks, J. Sun, M. M. Fergusson, J.C.J. Liu, K.M. Holmström, S. Menazza, D.A. Springer, C. Liu, B. Glancy, T. Finkel, E. Murphy, EMRE is essential for mitochondrial calcium uniporter activity in a mouse model, JCI Insight 5 (2020), https://doi.org/10.1172/JCI.INSIGHT.134063.
[91] A.N. Antony, M. Paillard, C. Moffat, E. Juskeviciute, J. Correnti, B. Bolon, E. Rubin, G. Csordás, E.L. Seifert, J.B. Hoek, G. Hajnóczky, MICU1 regulation of mitochondrial Ca(2+) uptake dictates survival and tissue regeneration, Nat. Commun. 7 (2016) 10955, https://doi.org/10.1038/ncomms10955.
[92] J.J.C.J. Liu, J.J.C.J. Liu, K.M. Holmström, S. Menazza, R.J. Parks, M. M. Fergusson, Z.-X.X. Yu, D.A. Springer, C. Halsey, C. Liu, E. Murphy, T. Finkel, MICU1 Serves as a Molecular Gatekeeper to Prevent In Vivo Mitochondrial Calcium Overload, Cell Rep 16 (2016) 1561–1573, https://doi.org/10.1016/j.celrep.2016.07.011.
[93] 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, A. Reghan Foley, G. Wheway, K. Szymanska, S. Natarajan, Z.A. Abdelhamed, J. E. Morgan, H. Roper, G.W.E Santen, E.H. Niks, W. Ludo 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, 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.
[94] A.G. Bick, H. Wakimoto, K.J. Kamer, Y. Sancak, O. Goldberger, A. Axelsson, D. M. DeLaughter, J.M. Gorham, V.K. Mootha, J.G. Seidman, C.E. Seidman, Cardiovascular homeostasis dependence on MICU2, a regulatory subunit of the mitochondrial calcium uniporter, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) E9096–E9104, https://doi.org/10.1073/PNAS.1711303114/SUPPL_FILE/PNAS.1711303114.SD06.XLSX.
[95] R.S. Balaban, The role of Ca2+ signaling in the coordination of mitochondrial ATP production with cardiac work, Biochim. Biophys. Acta - Bioenerg. 1787 (2009) 1334–1341, https://doi.org/10.1016/J.BBABIO.2009.05.011.
[96] E.J. Griffiths, G.A. Rutter, Mitochondrial calcium as a key regulator of mitochondrial ATP production in mammalian cells, Biochim. Biophys. Acta - Bioenerg. 1787 (2009) 1324–1333, https://doi.org/10.1016/J.BBABIO.2009.01.019.
[97] D.M. Bers, Cardiac excitation–contraction coupling, Nat. 415 (2002) 198–205, https://doi.org/10.1038/415198a, 2002 4156868.
[98] M.R. Duchen, O. McGuinness, L.A. Brown, M. Crompton, On the involvement of a cyclosporin A sensitive mitochondrial pore in myocardial reperfusion injury, Cardiovasc. Res. 27 (1993) 1790–1794, https://doi.org/10.1093/CVR/27.10.1790.
[99] E.J. Griffiths, A.P. Halestrap, Protection by Cyclosporin A of Ischemia/Reperfusion-Induced Damage in Isolated Rat Hearts, J. Mol. Cell. Cardiol. 25 (1993) 1461–1469, https://doi.org/10.1006/JMCC.1993.1162.
[100] A.E. Vercesi, R.F. Castilho, A.J. Kowaltowski, H.C.F. de Oliveira, N.C. de Souza-Pinto, T.R. Figueira, E.N.B. Busanello, Mitochondrial calcium transport and the redox nature of the calcium-induced membrane permeability transition, Free Radic, Biol. Med. 129 (2018) 1–24, https://doi.org/10.1016/J.FREERADBIOMED.2018.08.034.
[101] J.Q. Kwong, X. Lu, R.N. Correll, J.A. Schwanekamp, R.J. Vagnozzi, M.A. Sargent, A.J. York, J. Zhang, D.M. Bers, J.D. Molkentin, The Mitochondrial Calcium Uniporter Selectively Matches Metabolic Output to Acute Contractile Stress in the Heart, Cell Rep 12 (2015) 15–22, https://doi.org/10.1016/j.celrep.2015.06.002.
[102] T.S. Luongo, J.P. Lambert, A. Yuan, X. Zhang, P. Gross, J. Song, S. Shanmughapriya, E. Gao, M. Jain, S.R. Houser, W.J. Koch, J.Y. Cheung, M. Madesh, J.W. Elrod, The Mitochondrial Calcium Uniporter Matches Energetic Supply with Cardiac Workload during Stress and Modulates Permeability Transition, Cell Rep (2015), https://doi.org/10.1016/j.celrep.2015.06.017.
[103] T.P. Rasmussen, Y. Wu, M.A. Joiner, O.M. Koval, N.R. Wilson, E.D. Luczak, Q. Wang, B. Chen, Z. Gao, Z. Zhu, B.A. Wagner, J. Soto, M.L. McCormick, W. Kutschke, R.M. Weiss, L. Yu, R.L. Boudreau, E.D. Abel, F. Zhan, D.R. Spitz, G. R. Buettner, L.-S. Song, L.V Zingman, M.E. Anderson, Inhibition of MCU forces extramitochondrial adaptations governing physiological and pathological stress responses in heart, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 9129–9134, https://doi.org/10.1073/pnas.1504705112.
[104] Y. Wu, T.P. Rasmussen, O.M. Koval, M.-L.A. Joiner, D.D. Hall, B. Chen, E. D. Luczak, Q. Wang, A.G. Rokita, X.H.T. Wehrens, L.-S. Song, M.E. Anderson, The mitochondrial uniporter controls fight or flight heart rate increases, Nat. Commun. 6 (2015) 6081, https://doi.org/10.1038/ncomms7081.
[105] R.J. Parks, S. Menazza, K.M. Holmström, G. Amanakis, M. Fergusson, H. Ma, A. M. Aponte, P. Bernardi, T. Finkel, E. Murphy, Cyclophilin D-mediated regulation of the permeability transition pore is altered in mice lacking the mitochondrial calcium uniporter, Cardiovasc. Res. 115 (2019) 385–394, https://doi.org/10.1093/CVR/CVY218.
[106] A. Kosmach, B. Roman, J. Sun, A. Femnou, F. Zhang, C. Liu, C.A. Combs, R. S. Balaban, E. Murphy, Monitoring mitochondrial calcium and metabolism in the beating MCU-KO heart, Cell Rep 37 (2021) 109846, https://doi.org/10.1016/J.CELREP.2021.109846.
[107] K.M. Holmström, X. Pan, J.C. Liu, S. Menazza, J. Liu, T.T. Nguyen, H. Pan, R. J. Parks, S. Anderson, A. Noguchi, D. Springer, E. Murphy, T. Finkel, Assessment

of cardiac function in mice lacking the mitochondrial calcium uniporter, J. Mol. Cell. Cardiol. 85 (2015) 178–182, https://doi.org/10.1016/j.yjmcc.2015.05.022.
[108] Q. Xue, H. Pei, Q. Liu, M. Zhao, J. Sun, E. Gao, X. Ma, L. Tao, MICU1 protects against myocardial ischemia/reperfusion injury and its control by the importer receptor, Tom 70 (2017) 8, https://doi.org/10.1038/cddis.2017.280.
[109] T.R. Altamimi, Q.G. Karwi, G.M. Uddin, A. Fukushima, J.Q. Kwong, J. D. Molkentin, G.D. Lopaschuk, Cardiac-specific deficiency of the mitochondrial calcium uniporter augments fatty acid oxidation and functional reserve, J. Mol. Cell. Cardiol. 127 (2019) 223–231, https://doi.org/10.1016/J.YJMCC.2018.12.019.
[110] H. Chapoy Villanueva, J.H. Sung, J.A. Stevens, M.J. Zhang, P.M. Nelson, L. S. Denduluri, F. Feng, T.D. O'Connell, D.W. Townsend, J.C. Liu, Distinct effects of cardiac mitochondrial calcium uniporter inactivation via EMRE deletion in the short and long term, J. Mol. Cell. Cardiol. 181 (2023) 33–45, https://doi.org/10.1016/j.yjmcc.2023.05.007.
[111] V. Robert, P. Pinton, V. Tosello, R. Rizzuto, T. Pozzan, Recombinant aequorin as tool for monitoring calcium concentration in subcellular compartments, Methods Enzymol 327 (2000) 440–456, https://doi.org/10.1016/S0076-6879(00)27295-9.
[112] C.J. Bell, N.A. Bright, G.A. Rutter, E.J. Griffiths, ATP Regulation in Adult Rat Cardiomyocytes: TIME-RESOLVED DECODING OF RAPID MITOCHONDRIAL CALCIUM SPIKING IMAGED WITH TARGETED PHOTOPROTEINS, J. Biol. Chem. 281 (2006) 28058–28067, https://doi.org/10.1074/JBC.M604540200.
[113] K. Madsen, P. Ertbjerg, M.S. Djurhuus, P.K. Pedersen, Calcium content and respiratory control index of skeletal muscle mitochondria during exercise and recovery, Am. J. Physiol. 271 (1996) E1044–E1050, https://doi.org/10.1006/abio.1996.0197.
[114] E.R. Weibel, H. Hoppeler, Exercise-induced maximal metabolic rate scales with muscle aerobic capacity, J. Exp. Biol. 208 (2005) 1635–1644, https://doi.org/10.1242/jeb.01548.
[115] J.M. Argilés, N. Campos, J.M. Lopez-Pedrosa, R. Rueda, L. Rodriguez-Mañas, Skeletal Muscle Regulates Metabolism via Interorgan Crosstalk: Roles in Health and Disease, J. Am. Med. Dir. Assoc. 17 (2016) 789–796, https://doi.org/10.1016/J.JAMDA.2016.04.019.
[116] C. Mammucari, G. Gherardi, G. Lanfranchi, R. Rizzuto, I. Zamparo, A. Raffaello, S. Boncompagni, F. Chemello, S. Cagnin, A. Braga, S. Zanin, G. Pallafacchina, L. Zentilin, M. Sandri, D. De Stefani, F. Protasi, G. Lanfranchi, R. Rizzuto, The Mitochondrial Calcium Uniporter Controls Skeletal Muscle Trophism In Vivo, Cell Rep 10 (2015) 1269–1279, https://doi.org/10.1016/j.celrep.2015.01.056.
[117] G. Gherardi, L. Nogara, S. Ciciliot, G.P. Fadini, B. Blaauw, P. Braghetta, P. Bonaldo, D. De Stefani, R. Rizzuto, C. Mammucari, Loss of mitochondrial calcium uniporter rewires skeletal muscle metabolism and substrate preference, Cell Death Differ 26 (2018) 362–381, https://doi.org/10.1038/s41418-018-0191-7, 2018 262.
[118] J.Q. Kwong, J. Huo, M.J. Bround, J.G. Boyer, J.A. Schwanekamp, N. Ghazal, J. T. Maxwell, Y.C. Jang, Z. Khuchua, K. Shi, D.M. Bers, J. Davis, J.D. Molkentin, The mitochondrial calcium uniporter underlies metabolic fuel preference in skeletal muscle, JCI Insight 3 (2018), https://doi.org/10.1172/JCI.INSIGHT.121689.
[119] R. Sartori, V. Romanello, M. Sandri, Mechanisms of muscle atrophy and hypertrophy: implications in health and disease, Nat. Commun 12 (2021) 1–12, https://doi.org/10.1038/s41467-020-20123-1, 2021 121.
[120] J.L. Ruas, J.P. White, R.R. Rao, S. Kleiner, K.T. Brannan, B.C. Harrison, N. P. Greene, J. Wu, J.L. Estall, B.A. Irving, I.R. Lanza, K.A. Rasbach, M. Okutsu, K. S. Nair, Z. Yan, L.A. Leinwand, B.M. Spiegelman, A PGC-1α isoform induced by resistance training regulates skeletal muscle hypertrophy, Cell 151 (2012) 1319–1331, https://doi.org/10.1016/j.cell.2012.10.050.
[121] G. Butera, D. Vecellio Reane, M. Canato, L. Pietrangelo, S. Boncompagni, F. Protasi, R. Rizzuto, C. Reggiani, A. Raffaello, Parvalbumin affects skeletal muscle trophism through modulation of mitochondrial calcium uptake, Cell Rep 35 (2021) 109087, https://doi.org/10.1016/J.CELREP.2021.109087.
[122] V. Debattisti, A. Horn, R. Singh, E.L. Seifert, M.W. Hogarth, D.A. Mazala, K. T. Huang, R. Horvath, J.K. Jaiswal, G. Hajnóczky, Dysregulation of Mitochondrial Ca2+ Uptake and Sarcolemma Repair Underlie Muscle Weakness and Wasting in Patients and Mice Lacking MICU1, Cell Rep 29 (2019) 1274–1286, https://doi.org/10.1016/j.celrep.2019.09.063, e6.
[123] B. Roman, Y. Mastoor, Y. Zhang, D. Gross, D. Springer, C. Liu, B. Glancy, E. Murphy, Loss of mitochondrial Ca2+ uptake protein 3 impairs skeletal muscle calcium handling and exercise capacity, J. Physiol. 602 (2024) 113–128, https://doi.org/10.1113/JP284894.
[124] 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), https://doi.org/10.1212/NXG.0000000000000059.
[125] 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.
[126] K.M. Wilton, J.A. Morales-Rosado, D. Selcen, K. Muthusamy, S. Ewing, K. Agre, K. Nickels, E.W. Klee, M. Ho, E. Morava, Developmental brain abnormalities and acute encephalopathy in a patient with myopathy with extrapyramidal signs secondary to pathogenic variants in MICU1, JIMD Rep 53 (2020) 22–28, https://doi.org/10.1002/jmd2.12114.
[127] A. Horn, J.H. Van Der Meulen, A. Defour, M. Hogarth, S.C. Sreetama, A. Reed, L. Scheffer, N.S. Chandel, J.K. Jaiswal, Mitochondrial redox signaling enables repair of injured skeletal muscle cells, Sci. Signal. 10 (2017), https://doi.org/10.1126/SCISIGNAL.AAJ1978/SUPPL_FILE/AAJ1978_SM.PDF.
[128] 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, S. Alkuraya, A null mutation in MICU2 causes abnormal mitochondrial calcium homeostasis and a severe neurodevelopmental disorder, Brain 14 (2017) 403–410, https://doi.org/10.1093/brain/awx237.
[129] M.J. Amaya, M.H. Nathanson, Calcium Signaling in the Liver, Compr. Physiol. 3 (2013) 515–539, https://doi.org/10.1002/CPHY.C120013.
[130] E.A. Vilas-Boas, J.V. Cabral-Costa, V.M. Ramos, C.C. Caldeira da Silva, A. J. Kowaltowski, Goldilocks calcium concentrations and the regulation of oxidative phosphorylation: Too much, too little, or just right, J. Biol. Chem. 299 (2023) 102904, https://doi.org/10.1016/j.jbc.2023.102904.
[131] D. Tomar, F. Jaña, Z. Dong, W.J. Quinn, P. Jadiya, S.L. Breves, C.C. Daw, S. Srikantan, S. Shanmughapriya, N. Nemani, E. Carvalho, A. Tripathi, A. M. Worth, X. Zhang, R. Razmpour, A. Seelam, S. Rhode, A.V. Mehta, M. Murray, D. Slade, S.H. Ramirez, P. Mishra, G.S. Gerhard, J. Caplan, L. Norton, K. Sharma, S. Rajan, D. Balciunas, D.S. Wijesinghe, R.S. Ahima, J.A. Baur, M. Madesh, Blockade of MCU-Mediated Ca2+ Uptake Perturbs Lipid Metabolism via PP4-Dependent AMPK Dephosphorylation, Cell Rep 26 (2019) 3709–3725, https://doi.org/10.1016/J.CELREP.2019.02.107, e7.
[132] D.F.S. Rolfe, G.C. Brown, Cellular energy utilization and molecular origin of standard metabolic rate in mammals, Https://Doi.Org/10.1152/Physrev.1997.77.3.731 77 (1997) 731–758, https://doi.org/10.1152/PHYSREV.1997.77.3.731.
[133] G. Yellen, Fueling thought: Management of glycolysis and oxidative phosphorylation in neuronal metabolism, J. Cell Biol. 217 (2018) 2235–2246, https://doi.org/10.1083/JCB.201803152.
[134] E.G. Neal, H. Chaffe, R.H. Schwartz, M.S. Lawson, N. Edwards, G. Fitzsimmons, A. Whitney, J.H. Cross, The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial, Lancet Neurol 7 (2008) 500–506, https://doi.org/10.1016/S1474-4422(08)70092-9.
[135] G.E. Brancati, C. Rawas, A. Ghestem, C. Bernard, A.I. Ivanov, Spatio-temporal heterogeneity in hippocampal metabolism in control and epilepsy conditions, Proc. Natl. Acad. Sci. U. S. A. 118 (2021) e2013972118, https://doi.org/10.1073/PNAS.2013972118/SUPPL_FILE/PNAS.2013972118.SAPP.PDF.
[136] N.M. Márkus, P. Hasel, J. Qiu, K.F.S. Bell, S. Heron, P.C. Kind, O. Dando, T. I. Simpson, G.E. Hardingham, Expression of mRNA Encoding Mcu and Other Mitochondrial Calcium Regulatory Genes Depends on Cell Type, Neuronal Subtype, and Ca2+ Signaling, PLoS One 11 (2016) e0148164, https://doi.org/10.1371/JOURNAL.PONE.0148164.
[137] X.P. Cheng, A.Y. Vinokurov, E.A. Zherebtsov, O.A. Stelmashchuk, P.R. Angelova, N. Esteras, A.Y. Abramov, Variability of mitochondrial energy balance across brain regions, J. Neurochem. 157 (2021) 1234–1243, https://doi.org/10.1111/JNC.15239.
[138] E. Sjöstedt, W. Zhong, L. Fagerberg, M. Karlsson, N. Mitsios, C. Adori, P. Oksvold, F. Edfors, A. Limiszewska, F. Hikmet, J. Huang, Y. Du, L. Lin, Z. Dong, L. Yang, X. Liu, H. Jiang, X. Xu, J. Wang, H. Yang, L. Bolund, A. Mardinoglu, C. Zhang, K. von Feilitzen, C. Lindskog, F. Pontén, Y. Luo, T. Hökfelt, M. Uhlén, J. Mulder, An atlas of the protein-coding genes in the human, pig, and mouse brain, Science 367 (2020), https://doi.org/10.1126/science.aay5947.
[139] M. Karlsson, C. Zhang, L. Méar, W. Zhong, A. Digre, B. Katona, E. Sjöstedt, L. Butler, J. Odeberg, P. Dusart, F. Edfors, P. Oksvold, K. von Feilitzen, M. Zwahlen, M. Arif, O. Altay, X. Li, M. Ozcan, A. Mardonoglu, L. Fagerberg, J. Mulder, Y. Luo, F. Ponten, M. Uhlén, C. Lindskog, A single–cell type transcriptomics map of human tissues, Sci. Adv. 7 (2021), https://doi.org/10.1126/SCIADV.ABH2169/SUPPL_FILE/SCIADV.ABH2169_SM.PDF.
[140] O. Stoler, A. Stavsky, Y. Khrapunsky, I. Melamed, G. Stutzmann, D. Gitler, I. Sekler, I. Fleidervish, Frequency-and spike-timing-dependent mitochondrial Ca2+ signaling regulates the metabolic rate and synaptic efficacy in cortical neurons, Elife 11 (2022), https://doi.org/10.7554/ELIFE.74606.
[141] M. Nichols, P.A. Elustondo, J. Warford, A. Thirumaran, E.V. Pavlov, G. S. Robertson, Global ablation of the mitochondrial calcium uniporter increases glycolysis in cortical neurons subjected to energetic stressors, J. Cereb. Blood Flow Metab. 37 (2017) 3027–3041, https://doi.org/10.1177/0271678X16682250/ASSET/IMAGES/LARGE/10.1177_0271678X16682250-FIG7.JPEG.
[142] J. Qin, L. Liu, L. Liu, Z. Zhou, Y. Zhou, K. Zhang, B. Wang, H. Lu, J. Ran, T. Ma, Y. Zhang, Z. Li, X. Liu, The effect of regulating MCU expression on experimental ischemic brain injury, Exp. Neurol. 362 (2023) 114329, https://doi.org/10.1016/J.EXPNEUROL.2023.114329.
[143] M. Nichols, E.V. Pavlov, G.S. Robertson, Tamoxifen-induced knockdown of the mitochondrial calcium uniporter in Thy1-expressing neurons protects mice from hypoxic/ischemic brain injury, Cell Death Dis 9 (2018) 1–11, https://doi.org/10.1038/s41419-018-0607-9, 2018 96.
[144] V. Granatiero, M. Pacifici, A. Raffaello, D. De Stefani, R. Rizzuto, Overexpression of Mitochondrial Calcium Uniporter Causes Neuronal Death, Oxid. Med. Cell. Longev. (2019) 2019, https://doi.org/10.1155/2019/1681254.
[145] R. Singh, A. Bartok, M. Paillard, A. Tyburski, M. Elliott, G. Hajnóczky, Uncontrolled mitochondrial calcium uptake underlies the pathogenesis of neurodegeneration in MICU1-deficient mice and patients, Sci. Adv. 8 (2022) 4716, https://doi.org/10.1126/SCIADV.ABJ4716/SUPPL_FILE/SCIADV.ABJ4716_SOURCE_DATA.ZIP.

[146] S.E. Khoshnam, W. Winlow, M. Farzaneh, Y. Farbood, H.F. Moghaddam, Pathogenic mechanisms following ischemic stroke, Neurol. Sci. 38 (2017) 1167–1186, https://doi.org/10.1007/S10072-017-2938-1, 2017 387.
[147] A.Y. Abramov, M.R. Duchen, Mechanisms underlying the loss of mitochondrial membrane potential in glutamate excitotoxicity, Biochim. Biophys. Acta - Bioenerg. 1777 (2008) 953–964, https://doi.org/10.1016/J.BBABIO.2008.04.017.
[148] J. Qiu, Y.-W.W. Tan, A.M. Hagenston, M.-A.A. Martel, N. Kneisel, P.A. Skehel, D.J. A. a Wyllie, H. Bading, G.E. Hardingham, Mitochondrial calcium uniporter Mcu controls excitotoxicity and is transcriptionally repressed by neuroprotective nuclear calcium signals, Nat. Commun. 4 (2013) 2034, https://doi.org/10.1038/ncomms3034.
[149] G.A. Rutter, V. Sidarala, B.A. Kaufman, S.A. Soleimanpour, Mitochondrial metabolism and dynamics in pancreatic beta cell glucose sensing, Biochem. J. 480 (2023) 773–789, https://doi.org/10.1042/BCJ20230167.
[150] S.E. Kahn, M.E. Cooper, S. Del Prato, Pathophysiology and treatment of type 2 diabetes: Perspectives on the past, present, and future, Lancet 383 (2014) 1068–1083, https://doi.org/10.1016/S0140-6736(13)62154-6.
[151] A. Wiederkehr, G. Szanda, D. Akhmedov, C. Mataki, C.W. Heizmann, K. Schoonjans, T. Pozzan, A. Spät, C.B. Wollheim, Mitochondrial matrix calcium is an activating signal for hormone secretion, Cell Metab 13 (2011) 601–611, https://doi.org/10.1016/j.cmet.2011.03.015.
[152] M.R. Alam, L.N. Groschner, W. Parichatikanond, L. Kuo, A.I. Bondarenko, R. Rost, M. Waldeck-Weiermair, R. Malli, W.F. Graier, Mitochondrial Ca2+ uptake 1 (MICU1) and mitochondrial ca2+ uniporter (MCU) contribute to metabolism-secretion coupling in clonal pancreatic β-cells, J. Biol. Chem. 287 (2012) 34445–34454, https://doi.org/10.1074/jbc.M112.392084.
[153] A.I. Tarasov, F. Semplici, M.a. Ravier, E.a. Bellomo, T.J. Pullen, P. Gilon, I. Sekler, R. Rizzuto, G.a. Rutter, The Mitochondrial Ca2+ Uniporter MCU Is Essential for Glucose-Induced ATP Increases in Pancreatic β-Cells, PLoS One 7 (2012) e39722, https://doi.org/10.1371/journal.pone.0039722.
[154] E. Georgiadou, E. Haythorne, M.T. Dickerson, L. Lopez-Noriega, T.J. Pullen, G. da Silva Xavier, S.P.X. Davis, A. Martinez-Sanchez, F. Semplici, R. Rizzuto, J. A. McGinty, P.M. French, M.C. Cane, D.A. Jacobson, I. Leclerc, G.A. Rutter, The pore-forming subunit MCU of the mitochondrial Ca2+ uniporter is required for normal glucose-stimulated insulin secretion in vitro and in vivo in mice, Diabetologia 63 (2020) 1368–1381, https://doi.org/10.1007/s00125-020-05148-x.
[155] N. Vishnu, A. Hamilton, A. Bagge, A. Wernersson, E. Cowan, H. Barnard, Y. Sancak, K.J. Kamer, P. Spégel, M. Fex, A. Tengholm, V.K. Mootha, D. G. Nicholls, H. Mulder, Mitochondrial clearance of calcium facilitated by MICU2 controls insulin secretion, Mol. Metab. 51 (2021) 101239, https://doi.org/10.1016/J.MOLMET.2021.101239.
[156] A. De Mario, A. Tosatto, J.M. Hill, G. Szabadkai, R. Rizzuto, C. Mammucari, Identification and functional validation of FDA-approved positive and negative modulators of the mitochondrial calcium uniporter Graphical abstract Highlights d We screen an FDA-approved drug library for mitochondrial Ca 2+ uptake modulators d Amorolfine, CellReports 35 (2021) 109275, https://doi.org/10.1016/j.celrep.2021.109275.
[157] 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.
[158] 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.
[159] M. Rodríguez-Prados, K.T. Huang, K. Márta, M. Paillard, G. Csordás, S.K. Joseph, G. Hajnóczky, MICU1 controls the sensitivity of the mitochondrial Ca2+ uniporter to activators and inhibitors, Cell Chem. Biol. 30 (2023) 606–617, https://doi.org/10.1016/j.chembiol.2023.05.002, e4.