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Review

The MCU complex in cell death

Elisa Penna, Javier Espino, Diego De Stefani, Rosario Rizzuto*

Department of Biomedical Sciences, University of Padova, Italy

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ABSTRACT

During the 60s, the notion that positively charged Ca^{2+} ions are rapidly accumulated in energized mitochondria has been first established. In the following decades, mitochondrial Ca^{2+} homeostasis was shown to control cell metabolism, cell survival and other cell-specific functions through different mechanism. However, the molecular identity of the molecules controlling this process remained a mystery until just few years ago, when both mitochondrial Ca^{2+} uptake and release systems were genetically dissected. This finally opened the possibility to develop genetic model to directly test the contribution of mitochondrial Ca^{2+} homeostasis to cellular functions. Although the picture is still far from being clear, we here summarize and critically evaluate the current knowledge on how mitochondrial Ca^{2+} handling controls cell death.

1. Introduction

The divalent ion calcium (Ca^{2+}) acts as a second messenger in multiple signal-transduction pathways, thereby controlling several biological processes in the cell. Within this context, a fundamental role is attributed to mitochondrial Ca^{2+} uptake [1]. First, it is well-established that Ca^{2+} accumulation within mitochondria activates three Krebs cycle dehydrogenases, i.e., pyruvate, α-ketoglutarate and isocitrate dehydrogenases, thus modulating ATP production by oxidative phosphorylation [2,3]. Second, mitochondria can withdraw large amounts of Ca^{2+} from a defined subcellular domain, hence shaping cytosolic Ca^{2+} signals and influencing cell function [4,5]. Third, mitochondrial Ca^{2+} overload can trigger either apoptotic or necrotic cell death and therefore the uptake of Ca^{2+} by mitochondria must be tightly controlled [6]. Last, mitochondrial Ca^{2+} is also involved in the regulation of autophagy, which is in turn activated when endoplasmic reticulum (ER)-to-mitochondria Ca^{2+} transfer is compromised [7].

Mitochondrial Ca^{2+} homeostasis is finely tuned by several channels, exchangers and regulatory subunits, whose molecular identity has been unveiled during the last few years. Herein, we first summarize the main mechanisms engaged in Ca^{2+} transport across the inner mitochondrial membrane (IMM). Then, we review recent progress in the characterization of the mitochondrial calcium uniporter (MCU) complex, a macromolecular structure that mediates Ca^{2+} influx into the organelle matrix, emphasizing the functional role of each component of the complex, its putative structure and the regulation of its expression. Finally, we focus on the (patho)physiological relevance of the MCU complex as a master regulator of cell death.

2. Mitochondrial Ca^{2+} transport

Mitochondria are endowed with the appropriate machinery to transport Ca^{2+} ions into and out of the organelle matrix. In fact, it has long been known that the influx pathway is assured by a uniport mechanism, the so-called Ca^{2+} uniporter, which is driven by the mitochondrial membrane potential generated by the electron transport chain [8,9]. On the contrary, mitochondria release Ca^{2+} through two different antiport processes, e.g., exchanging Ca^{2+} with H^+ (as established in liver) or with Na^+ (as demonstrated in excitable tissues) [10,11]. Nevertheless, the molecular identification of mitochondrial Ca^{2+} transporters involved in the influx and efflux pathways has represented a tough challenge for researchers in this field for about 50 years.

The recent discovery of the mitochondrial Na^+/Ca^{2+} exchanger, referred to as Na^+/Ca^{2+} Li^+-permeable exchanger (NCLX), meant a milestone in mitochondrial research [12]. NCLX catalyzes the exchange of 3 or 4 Na^+ per Ca^{2+}, thereby confirming that the mitochondrial Na^+/Ca^{2+} antiport is electrogenic, with a net import of one or two positive charges into the matrix. NCLX-mediated mitochondrial Ca^{2+} efflux is also stimulated by Li^+ (not only by Na^+), which is a distinctive attribute of mitochondrial Na^+-dependent Ca^{2+} extrusion [13,14]. Interestingly, NCLX was shown to be confined to the IMM and reported to form 100-kDa dimers [12], the size being pretty similar to that previously found in molecularly unidentified mitochondrial complexes (i.e., ~110 kDa) depicting Na^+/Ca^{2+} exchange activity [15,16].

The H^+/Ca^{2+} exchange system has been for the most part characterize in isolated mitochondria and is considered electroneutral (2

* Corresponding author at: Contact information: Department of Biomedical Sciences, University of Padova, Via Ugo Bassi 58/B, 35131, Padova, Italy.
E-mail address: rosario.rizzuto@unipd.it (R. Rizzuto).

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H^+ per Ca^{2+}) [17]. The molecular nature of the H^+/Ca^{2+} antiporter however remains to be deciphered. Recently, leucine zipper-EF-hand containing transmembrane protein 1 (LETM1) has been put forward as a feasible candidate [18]. By expressing purified LETM1 in liposomes, Miller and coworkers demonstrated that such protein catalyzes H^+/Ca^{2+} antiport independently of K^+. Nevertheless, previous research initially pointed out its role as a mitochondrial K^+/H^+ exchanger [19–21], and therefore the function of LETM1 in mitochondrial physiology needs to be further corroborated.

Mitochondria take up Ca^{2+} through a highly-selective Ca^{2+}-conducting channel, termed Mitochondrial Calcium Uniporter, thanks to the huge driving force represented by the mitochondrial membrane potential, which is generated by the pumping of H^+ towards the intermembrane space by the electron transport chain. MCU is characterized by a low Ca^{2+} affinity (K_D = 20–30 μM), thereby suggesting that high Ca^{2+} concentration is required to activate the channel [22], although the activation threshold is significantly lower in some tissues [23,24]. Nevertheless, physiological stimulation can trigger a steep increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) up to approximately 1 μM, a fact that may pose discrepancies due to the low Ca^{2+} affinity of the MCU. This peculiarity is explained by the demonstration that high $[Ca^{2+}]_{cyt}$ (> 10 μM) microdomains can form transiently in regions of close apposition between mitochondria and the Ca^{2+} channels of the ER (or plasma membrane) [25–29]. Therefore, the MCU (and hence mitochondria) senses a microdomain of high $[Ca^{2+}]_{cyt}$ at the mouth of open ER (or plasma membrane) channels and rapidly accumulates Ca^{2+} into the organelle matrix, thus dissipating the $[Ca^{2+}]_{cyt}$ microdomain.

The identification of the long-sought molecular players involved in mitochondrial Ca^{2+} uptake began with the completion of the mammalian mitochondrial gene catalogue MitoCarta [30], which allowed scientists in the field to identify the first uniporter component in 2010, namely mitochondrial calcium uptake protein 1 (MICU1; also known as CBARA1 and EFHA3) [31]. These fundamental findings paved the way for the discovery of the pore-forming subunit of the MCU complex, i.e., MCU itself (also known as CCDC109A), one year later by two independent research groups [32,33]. Thereafter, intensive research has led to the identification of other constituents such as MCU regulatory subunit b (MCUb; also known as CCDC109B) [34], MICU2 (also known as EFHA1 [35] and essential MCU regulator (EMRE; also known as C22orf32) [36], thereby adding various levels of complexity to the structure and regulation of the uniporter (see the section on *Bona fide* components of the MCU complex).

3. The MCU complex

The following section is subdivided to discriminate between the genuine components of the complex, on one hand, and its associated regulators, on the other hand.

3.1. *Bona fide components of the MCU complex*

The core component of the complex remained elusive until the identification in 2011 of a 40-kDa protein in the IMM, namely MCU [32,33]. It is encoded by the nuclear gene *CCDC109A*, which present homologs in virtually all plants and metazoans, but it is absent in yeast and specific protozoan and fungi branches [37,38]. MCU possesses two coiled-coil domains and two transmembrane (TM) helices separated by a highly-conserved short loop rich in acidic residues (EYSWDIMEP), which contains a four-residue DIME motif that most likely bestows MCU with Ca^{2+} selectivity [32,33]. Indeed, substitution of the two negatively charged residues in the DIME motif (D261/E264) abolishes MCU activity [32,33]. Regarding the topology, the linker faces the intermembrane space and is flanked by an N-terminal domain and C-terminal domain located within the mitochondrial matrix [39]. Both biochemical and computational data provide evidence that MCU can oligomerize within the IMM, thereby giving rise to a multimeric channel [34]. The predicted quaternary structure was initially predicted to be a tetramer made up of eight TM helices that create the pore across the membrane [34]; however, this view has been recently challenged and a different stoichiometry of the quaternary structure has been suggested (see the section on Structure of MCU complex) [40].

Compelling evidence supports the notion that MCU is the pore-forming subunit of the MCU complex. Thus, knockdown of MCU has proven to largely blunt mitochondrial Ca^{2+} entry in both live cells and whole-mitoplasts [32,33,41], an observation that held true in several cell types, including cardiomyocytes [42], pancreatic β-cells [43,44] and neurons [45]. Besides, mitochondria isolated from different tissues of MCU knockout mice failed to take up Ca^{2+} [46]. Accordingly, the overexpression of MCU alone enhances mitochondrial Ca^{2+} uptake in live cells [32] and increases mitochondrial Ca^{2+} current in isolated mitoplasts [41]. The latter findings account for the assumption that MCU on its own is able to form a functional Ca^{2+} channel, a view that is also supported by data in *in vitro* lipid bilayers [32,34]. However, an additional subunit named EMRE is needed to form a functional channel in vivo in metazoans (see later), likely indicating that channel formation could differ in synthetic vs endogenous membranes.

Subsequent studies revealed that MCU was only the tip of the iceberg. In fact, homology analysis soon after allowed the identification of an MCU paralog that was termed MCUb [34]. It is a 33-kDa highly conserved protein that bears 50% similarity to MCU. MCUb and MCU share similar structure (two coiled-coil domains, two TM helices connected through a short linker) and the same membrane topology, but they slightly differ in the loop region (VYSWDIMEP; note the amino acid substitution E256 V). Moreover, MCUb is less abundant than MCU and presents a differential expression pattern [34]. Thus, for instance, MCUb mRNA is highly expressed in heart and lung, whereas scarcely expressed in skeletal muscle [34]. Importantly, this variation in the ratio MCU:MCUb (3:1 and 40:1 in heart and skeletal muscle, respectively) correlates with recent findings indicating that the overall activity of the MCU complex is tissue-dependent (Ca^{2+} current is 30-fold larger in skeletal muscle than in heart mitochondria) [47].

From a functional point of view, MCUb overexpression causes a reduction in agonist-stimulated mitochondrial Ca^{2+} uptake, while its silencing induces the opposite effects, i.e., a significant increase. Reconstitution of recombinant MCUb in artificial membranes does not result in channel activity in calcium; however, co-expression of MCU and MCUb, which can form hetero-oligomers, dramatically decreases MCU activity in lipid bilayers [34]. Altogether, these data indicate that, when inserted in the channel, MCUb behaves as a dominant-negative subunit, thereby affecting the Ca^{2+} permeation properties of the uniporter [34].

A third fundamental component of the MCU complex, namely EMRE, was identified by taking advantage of mass spectrometry-based quantitative proteomics [48]. It is a small (10 kDa) IMM protein with a unique TM domain and a highly conserved, aspartate-enriched C-terminal region. In mammals, MCU-EMRE interaction is apparently necessary for *in vivo* uniporter activity [48,49]. Likewise, EMRE has been also reported to interact with MICU1 (an essential regulator of the MCU complex activity, as discussed below) [48,50], thus connecting the calcium-sensing with the calcium-conducting moieties of the uniporter. Paradoxically, EMRE distribution is confined to metazoans, whilst there are no homologs of EMRE in plants, protozoa and fungi [38,48], wherein MCU and MICU1 are widely conserved. In this line, it was established that both MCU and EMRE are required for the activity of metazoan MCU complex, but this does not hold true for *Dictyostelium Discoideum*, in which MCU *per se* is able to sustain uniporter activity [49]. Nevertheless, EMRE's bridging function is clearly in contrast with both the demonstration that MCU itself is sufficient to form a Ca^{2+} channel [32] and the activating capability of MICU1 on MCU [51] in planar lipid bilayers. Therefore, despite the fact that EMRE may have evolved in animal cells to improve complex assembly (hence enhancing