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Molecular regulation of MCU: Implications in physiology and disease

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ABSTRACT

Ca^{2+} flux across the inner mitochondrial membrane (IMM) regulates cellular bioenergetics, intra-cellular cytoplasmic Ca^{2+} signals, and various cell death pathways. Ca^{2+} entry into the mitochondria occurs due to the highly negative membrane potential ($\Delta\Psi_m$) through a selective inward rectifying MCU channel. In addition to being regulated by various mitochondrial matrix resident proteins such as MICUs, MCUb, MCUR1 and EMRE, the channel is transcriptionally regulated by upstream Ca^{2+} cascade, post transnational modification and by divalent cations. The mode of regulation either inhibits or enhances MCU channel activity and thus regulates mitochondrial metabolism and cell fate.

Ca^{2+} ion is a versatile second messenger essential for a variety of kinetically different cellular processes from fertilization to cell death [1]. While some processes like endocytosis occur in seconds, other processes such as gene transcription take up to hours and how Ca^{2+} regulates these diverse processes is a question still being studied [2]. A rise in cytosolic Ca^{2+} ($_cCa^{2+}$) can occur either by Ca^{2+} entry through plasma membrane channels (voltage gated/receptor mediated/second messenger mediated channels/store operated channels) or release from intracellular Ca^{2+} stores [3]. One ubiquitous mode of receptor-regulated Ca^{2+} entry is capacitative calcium entry, first proposed by Putney [4]. At any given point, resting $_cCa^{2+}$ is kept low (~ 100 nM) and this is achieved by Ca^{2+} efflux mechanisms of pumps (PMCA), exchangers (NCX) and organelles such as ER and the mitochondria that act as Ca^{2+} sinks. $_cCa^{2+}$ transients are defined and shaped by the mitochondria. The first observation of mitochondrial Ca^{2+} ($_mCa^{2+}$) uptake was evidenced five decades ago when several groups witnessed isolated mitochondria to buffer Ca^{2+} [5]. Since then, $_mCa^{2+}$ has been extensively studied. Three main roles have been attributed to Ca^{2+} uptake by the mitochondria: 1) Ca^{2+} is utilized by the dehydrogenases of the TCA cycle (pyruvate dehydrogenase, iso-citrate dehydrogenase and α-ketoglutarate dehydrogenase) for ATP generation. 2) To maintain $_cCa^{2+}$ dynamics. 3) Activation of various cell death pathways of apoptosis and necrosis [6–10].

Ca^{2+} entry into the mitochondria is due to the high electrochemical gradient (~ −180 mV) and occurs without the transport of any other ions hence making it a uniporter. Studies on isolated mitochondria estimated a flux of more than 10,000 Ca^{2+} ions per second through the channel [5]. Although the properties of the uniporter were established, its molecular identity remained elusive. The identification of the mitochondrial calcium uniporter (MCU) was made possible with the advances in integrative genomics [11,12]. Progress in the field has been made in identifying and characterizing MCU and its regulatory molecules.

Here, in this review we summarize the architecture of MCU channel, its mitochondrial resident regulators, regulation of the channel by ions and redox molecules and go onto explore the activity of the channel in pathological conditions along with therapeutic insights.

1. Architecture of MCU

Early work on $_mCa^{2+}$ uptake revealed that Ca^{2+} uptake into the mitochondria was $\Delta\Psi_m$ dependent with no transport of anions hence making it a uniporter. The transport of Ca^{2+} followed second order kinetics and was sensitive to Ruthenium Red (RR) (an inorganic cationic dye that binds acidic mucopolysaccharides and phospholipids) [13,14]. Electrophysiological studies defined the channel to be an

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inward rectifying current with high specificity for divalent cations Ca^{2+} $\sim Sr^{2+} > > Mn^{2+} \sim Ba^{2+}$ and to be inhibited at Nano molar concentrations of RR and its analog Ru360 [15]. Although the biophysical properties of the channel were characterized as being 1. Electrogenic dependent 2. Selective and 3. Low affinity for Ca^{2+}, it was not until 2011 when two independent groups discovered the molecular identity of the channel. Whole genome phylogenetic profiling, RNA co-expression analysis and organelle wide protein co-expression analysis revealed an RR sensitive transmembrane protein resident of the IMM that is part of a large complex called the MCU [11,12]. Reconstitution of MCU in planar lipid bilayer produced channel recordings with conductance similar to previously findings. Additionally, presence of RR failed to produce Ca^{2+}-permeable channel activity indicating sensitivity to ruthenium, a characteristic of the $_{m}Ca^{2+}$ transport [12]. Consistent with the finding that all vertebrate mitochondria take up Ca^{2+}, the expression of MCU is conserved across eukaryotes except in yeast. In contrast to their evolutionary sister group Amoebozoa that have a single homolog of MCU and MICU1, yeast do not exhibit uniporter activity with no homologs of the uniporter components [16]. Because of this property yeast serve as an excellent heterologous expression system. Reconstitution of DdMCU (MCU from Dictyostelium discoideum) in yeast was sufficient to evoke a $_{m}Ca^{2+}$ uptake response that was otherwise absent, further suggesting that MCU was the pore-forming subunit of the uniporter complex. MCU, the pore forming channel has been identified to be a two transmembrane, Ca^{2+} selective, ruthenium sensitive channel with its selectivity filter in the intermembrane space (IMS) and N and C terminal domains resting in the mitochondrial matrix [16] (Fig. 1).

The structure of N-terminal domain (NTD) of MCU spanning exons 3 and 4 adopts a β-grasp like fold that entails an $\alpha-$helix and six β-strands that form the central core with two highly conserved leucine rich loops [17]. Atomic resolution structure of NTD revealed a cluster of negatively charged residues called the MCU-regulating acidic patch (MRAP) in the β-grasp fold domain that binds divalent cations. Either interaction of Ca^{2+} or Mg^{2+} with the MRAP domain or mutations in the MRAP domain destabilizes MCU and shifts the self-association equilibrium to monomer with a loss of $_{m}Ca^{2+}$ uptake. This study for the first time shows that like most Ca^{2+} channels that are regulated by Ca^{2+} feedback mechanisms, MCU is autoregulated by matrix Ca^{2+} and Mg^{2+} binding to the MRAP domain in the NTD of MCU [18]. Expression of $MCU^{\Delta NTD}$ localizes to the mitochondria, and forms MCU oligomers with intact $\Delta\Psi_m$. Deletion of the NTD showed a significant reduction in $_{m}Ca^{2+}$ uptake with intact $_{c}Ca^{2+}$ dynamics. Co-immuno precipitation assay revealed that loss of NTD had diminished interaction with MCUR1 (a positive regulator of MCU) but not MICU1 and MICU2 [17]. The C-terminal domain (CTD) harbors two transmembrane domains and two coiled-coiled domains that are required for interaction with its regulators. Nuclear magnetic resonance (NMR) in combination with electron microscopy revealed the architecture of the MCU channel to be a pentamer. The second transmembrane (TM2) and the coiled-coil helix (CCH) form the inner cores of the pentamer that are wrapped by the first TM helix and CCH respectively. TM1 and TM2 domains are connected by a loop consisting of conserved acidic residues called the DIME motif loop that forms the pentameric barrel at the mouth of the pore. Asp240 and Glu243 are positioned inside the barrel to form two carboxylate rings that make up the selectivity filter of the channel [19]. Although this finding for the first time reported the structure of the *C.elegans* MCU pore, no proof of channel activity was performed in the pentameric state.

2. Regulators of MCU channel

MCU exists as part of a heteromeric complex that consist of MICU1, MICU2, MCUR1, EMRE, MCUb and SLC25A23 [20–24]. Sequence analysis of MCU identified a gene with 50% similarity to MCU called MCUb. MCUb is conserved across all vertebrates and absent in species of plants, kinetoplastids, Nematoda, and Arthropoda where MCU is present. Immuno-precipitation and foster resonance energy transfer (FRET) analysis revealed interaction of MCU and MCUb. However, electrophysiological studies of purified MCUb inserted into planar lipid bilayers showed no channel activity. While RNAi mediated silencing of MCUb in HeLa cells demonstrated an increase in $_{m}Ca^{2+}$ uptake upon stimulation with histamine, over-expression of MCUb resulted in a marked decrease in $_{m}Ca^{2+}$ uptake. These pieces of data indicate that MCUb functions as a dominant negative MCU sub-unit [25].

The activity of the channel is determined by its negative regulator MICU1 and its positive regulator MCUR1. Like most ion channels that open upon agonist stimulation, MCU channel opens only when $_{c}Ca^{2+}$ rises above 1–2 μM. The threshold is set by its gate-keeper MICU1. The EF hands of MICU1 sense the rise in $_{c}Ca^{2+}$ (> 3 μM) and physically dissociate from the channel thus transforming MCU to its open conformation [26]. MICU1 was the first MCU uniplex component to be identified using targeted RNAi screen based on clues from comparative physiology, evolutionary genomics and organelle proteomics. MICU1 was found to be an IMS resident protein with its EF hand domains facing the cytosol. It was also shown that silencing MICU1 abrogated

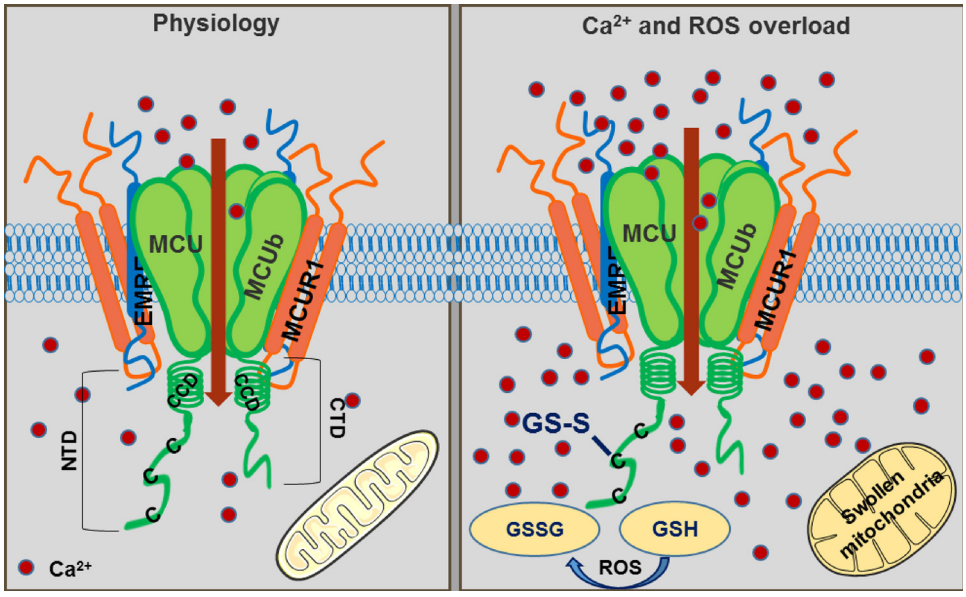

Fig. 1. MCU super complex in physiology and pathology.
Ca^{2+} enters the mitochondria through MCU channel. MCU, the pore-forming subunit is a pentamer with N and C terminal domains in the matrix. MCUR1 and EMRE are transmembrane proteins that interact with MCU and regulate $_{m}Ca^{2+}$ uptake by the channel. Under conditions of oxidative stress, C97 at the NTD of MCU gets S-glutathionylated promoting MCU oligomerization and increasing $_{m}Ca^{2+}$ uptake leading to Ca^{2+} overload and swelling of the mitochondria.

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