

Invited Mini Review

Structural basis of Ca^{2+} uptake by mitochondrial calcium uniporter in mitochondria: a brief review

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Mitochondria are cellular organelles that perform various functions within cells. They are responsible for ATP production, cell-signal regulation, autophagy, and cell apoptosis. Because the mitochondrial proteins that perform these functions need Ca^{2+} ions for their activity, mitochondria have ion channels to selectively uptake Ca^{2+} ions from the cytoplasm. The ion channel known to play the most important role in the Ca^{2+} uptake in mitochondria is the mitochondrial calcium uniporter (MCU) holo-complex located in the inner mitochondrial membrane (IMM). This ion channel complex exists in the form of a complex consisting of the pore-forming protein through which the Ca^{2+} ions are transported into the mitochondrial matrix, and the auxiliary protein involved in regulating the activity of the Ca^{2+} uptake by the MCU holo-complex. Studies of this MCU holo-complex have long been conducted, but we didn't know in detail how mitochondria uptake Ca^{2+} ions through this ion channel complex or how the activity of this ion channel complex is regulated. Recently, the protein structure of the MCU holo-complex was identified, enabling the mechanism of Ca^{2+} uptake and its regulation by the MCU holo-complex to be confirmed. In this review, I will introduce the mechanism of action of the MCU holo-complex at the molecular level based on the Cryo-EM structure of the MCU holo-complex to help understand how mitochondria uptake the necessary Ca^{2+} ions through the MCU holo-complex and how these Ca^{2+} uptake mechanisms are regulated. [BMB Reports 2022; 55(11): 528-534]

INTRODUCTION

All cells have a cell membrane, which separates the outside from the inside of the cell. By numerous metabolic processes, cells must take in the materials they need, and they must also expel the substances they make that are undesired, often by

means of the proteins in the cell membrane that surrounds the cell.

Ion channels are typical proteins that are involved in the transport of substances in the cell membrane (1). Since many ions are needed for the various metabolic processes taking place inside the cell, the cell must take in the requisite ions from the environment and release them to the environment if there are too many ions inside. However, ions cannot traverse hydrophobic cell membranes by themselves, because of their high hydrophilicity. In order to have only the right number of ions in the cell, cells use ion channels in the cell membrane to absorb the required ions and release ions that are no longer required or that are present in excess.

Ion channels in the cell membrane allow Ca^{2+} ions, which are representative ions, to pass across the membrane. Ca^{2+} ions are a type of second messenger; that is, they are a typical signal-transmission material that carries signals generated in cells (2-5). For example, Ca^{2+} ions in nerve cells serve as neurotransmitters (6), and Ca^{2+} ions in muscle cells are responsible for transmitting signals that cause muscles to contract. Ca^{2+} ions also play a significant role in the regulation of the activity of enzymes. This is accomplished by the Ca^{2+} ion's role as co-factors in a variety of enzymes that are responsible for mediating different metabolic events inside cells. Ca^{2+} ions are needed for clotting, and a variety of enzymes that mediate clotting events require Ca^{2+} ions as key coenzymes in order to cause clotting reactions (7). Ca^{2+} ions are found in bones and teeth, also play a role in the construction of the organs in the body, and are necessary for the production of bone and tooth. Most of the Ca^{2+} present in the body resides as calcium hydroxyapatite in the bones and teeth (8).

Most eukaryotic cells contain mitochondria, which are essential parts of a cell. The energy needed for cell growth is produced by mitochondria during cellular respiration in the form of adenosine triphosphate (ATP) (9, 10). And mitochondria also affect programmed cell death and the production of reactive oxygen species (ROS), which in turn regulate cell signaling (11-14). Finally, mitochondria and endoplasmic reticulum (ER) are in charge of controlling the level of Ca^{2+} ions in cells by storing Ca^{2+} ions (15-19).

Ca^{2+} ions are needed for the functions of mitochondria as cell organelles. In mitochondria, for example, ATP is synthesized by means of the TCA cycle (also known as the citric-acid cycle

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or the Krebs cycle), which consists of a chain reaction of several enzymes. The efficiency of ATP synthesis is affected by Ca^{2+} concentrations in the mitochondria because some of the enzymes in the TCA cycle require Ca^{2+} ions to be active (9, 10). On the other hand, excessive Ca^{2+} concentration in the mitochondria activates the pro-apoptotic factor, ultimately causing apoptosis (11, 20-22). These examples indicate that Ca^{2+} ions in mitochondria are very important for their functions in cells, and ultimately reflect that maintaining the Ca^{2+} concentration in mitochondria is essential for normal cell metabolism.

Since it is important to maintain Ca^{2+} concentrations in mitochondria, many researchers have wondered how mitochondria absorb and release Ca^{2+} ions to control their concentrations. Because mitochondria have a double membrane structure, ion channels and transporters for Ca^{2+} migration are required in the mitochondrial membrane. Therefore, many researches have long focused on finding proteins involved in Ca^{2+} migration across the mitochondrial membrane. Mitochondrial sodium-calcium exchangers (mNCLX) and mitochondrial proton-calcium exchangers (mHCX) are proteins that have been identified by these studies (23-25). They are transporters located in the IMM and play a role in releasing Ca^{2+} ions accumulated in the mitochondrial matrix into the mitochondrial intermembrane space (IMS) mediated by Na^+ and H^+ . In contrast, proteins that uptake Ca^{2+} ions from IMS into the mitochondrial matrix are also known. One such is, like mHCX and mNCLX, in the IMM and is known to act very selectively on Ca^{2+} ions. Unlike mHCX and mNCLX, however, the protein acts as a uniporter, transferring Ca^{2+} ions only to the mitochondrial matrix, and is selectively inhibited by inhibitors such as Ruthenium red (RuR) (26). This protein was later identified as a mitochondrial calcium uniporter (MCU) holo-complex, which consists of the MCU, an ion-conducting pore protein with a Ca^{2+} -selective filter and a luminal gate to regulate Ca^{2+} entry into the mitochondrial matrix, MICU1 and MICU2 that sense Ca^{2+} concentrations in the IMS, and EMRE (essential MCU regulator), which simultaneously binds to the MCU and MICU1 to regulate the activity of the MCU holo-complex (27-31). In this review, I will explain how mitochondria selectively uptake Ca^{2+} ions through the MCU holo-complex and how this Ca^{2+} uptake mechanism is regulated based on the structure of the MCU holo-complex.

MCU, ACTS AS A Ca^{2+} CHANNEL THAT CAN CONTROL THEIR ACTIVITY BY THEMSELVES

The MCU is the pore-forming part in the MCU holo-complex and is responsible for transferring Ca^{2+} ions from the IMS to the mitochondrial matrix across the IMM (32). According to genome sequence analysis, the MCU holo-complex of all metazoans, including humans, contains MCUs, which also exist in non-metazoan organisms, such as fungi (32, 33). That is, the MCU is the most essential subunit in the MCU holo-complex of all living things.

To date, the MCU structure of four fungal MCUs and one C.

elegans MCU has been identified through Cryo-EM, X-ray crystallography, and NMR, and these structures help us understand how mitochondria uptake Ca^{2+} ions through MCUs at the molecular level (34-38).

The MCU consists of three parts: N-terminal domain (NTD), coiled-coil domain (CCD), and transmembrane domain (TMD) (Fig. 1A) (34-37). In the MCU, the NTD is exposed to the mitochondrial matrix, the TMD is present in the IMM, and the CCD is located between these two domains. The MCU has a tetrameric structure in which pores are formed in the TMD to create a path through which Ca^{2+} ions can move. In the MCU, the TMD represents a four-fold symmetry, which is effective in transferring Ca^{2+} ions through a pore made by a tetrameric conformation of TMD (Fig. 1B) (34-37). The TMD of the MCU protomer consists of two α -helix (TMH: transmembrane helix). In the tetrameric structure of the MCU, TMH2 enters inward to form a pore, whereas TMH1 is exposed to the outside to contact the membrane (Fig. 1B). In the TMD, a DxxE motif (x are hydrophobic residues randomly) is located at the entrance side of the pore into which Ca^{2+} ions are introduced (Fig. 2A, B) (34-37). This DxxE motif is the most representative motif that characterizes MCUs and is conserved in the MCUs of all species as found by genome sequence analysis (Fig. 2C) (27, 28). That is, this DxxE motif plays a very important role in the function of the MCU. In the MCU structure, the function of this DxxE motif can be understood in more detail as a selective filter. The DxxE motif is located at TMH2 (Fig. 2A, B) (34-37). This motif has two acidic amino acids (Asp and Glu), which allow the MCU to have high selectivity for Ca^{2+} ions as well as uptake of Ca^{2+} . Glu plays a key role in making the MCU highly selective for Ca^{2+} ions. Because of the tetrameric arrangement of the MCU protomer, Glu is also arranged in four-fold symmetry, and Ca^{2+} ions coordinate with each Glu at the center of the arrangement, allowing the MCU to transport Ca^{2+} ions (Fig. 2B) (34-37). What is most characteristic here is the dis-

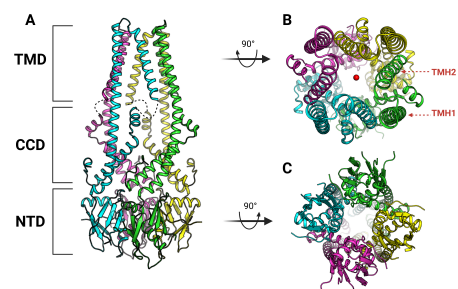


Fig. 1. Overall architecture of Fungal MCU of *N. crassa* (37). (A) Each domains of *N. crassa* MCU are indicated (PDB code: 6DT0). Each protomer are colored separately. Missing part of *N. crassa* MCU structure are presented by black dashes. (B) Top view of *N. crassa* MCU structure. Ca^{2+} are represented by red spheres. Each TMH is marked in red letters at single protomer. (C) Bottom view of *N. crassa* MCU structure.

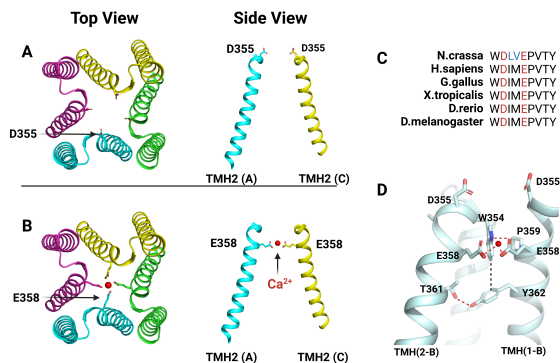


Fig. 2. Ca²⁺ Selective filter in *N.crasca* MCU. (A) Top and side view of Asp (D355) ring in the pore of *N.crasca* MCU. It's shown that side chain of D355 is in the shape of facing IMS at a side view. (B) Top and side view of Glu (E358) ring in the pore of *N.crasca* MCU. At top view, it's shown clearly that side chain of E358 is toward to Ca²⁺ (red circle) to coordinate them. (C) Sequence alignment of DxxE motif from human to fungi. The two acidic residues in DxxE motif are represented in red and the two residues in cyan are those that exist only in the *N.crasca* MCU in DxxE motif. (D) Interactions made between DxxE motif and neighboring residues; hydrogen bonds are marked with red dashes and van der Waals interaction is marked with black dash.

tance between Glu and Glu, which represents the minimum distance that can make an ionic interaction with Ca²⁺ ions only; so Ca²⁺ ions can pass selectively through the Glu ring composed of four Glu (Fig. 2B). On the other hand, the Asp of the DxxE motif is exposed to the IMS and forms a ring wider than the Glu ring (Fig. 2A) (34-37). Therefore, rather than acting like Glu as a filter for Ca²⁺ ions, Asp plays a role in increasing the Ca²⁺ uptake efficiency of mitochondria through the MCU by recruiting Ca²⁺ ions from the IMS. Interestingly, RuR and Ru360—inhibitors that selectively act on the MCU—bind to the MCU via Asp (39). Considering the position of Asp in the MCU structure, RuR or Ru360 binds to the Asp ring and physically blocks the access of the Ca²⁺ ions to the entrance of the pore of the MCU, thereby inhibiting this activity of the MCU. In addition, MICU1 competes with RuR and Ru360 to inhibit their activity; hence the Asp of the DxxE motif plays an important role in binding to MICU1 (40).

Although residues consisting of the DxxE motif are important residues for the Ca²⁺ uptake of the MCU, the residue around the DxxE motif has also significant role. In fact, in all MCUs, not only the DxxE motif but also all the residues around this motif are conserved (Fig. 2C). Their roles are shown in Fig. 2D in detail. Thr of TMH2 makes hydrogen bonds with Tyr in TMH1 of the neighboring protomer. Trp of TMH2 interacts with this Tyr, and at the same time has a stacking interaction with the Pro of TMH1 in the neighboring protomer. Trp can thus interact with Glu in the DxxE motif. This interaction directs the side chain of Glu toward the center of the pore, allowing it to bind with Ca²⁺ ions entering the pore. Therefore,

residues around the DxxE motif facilitate the binding of Glu in the DxxE motif with Ca²⁺ ions, ultimately helping Glu to act as a Ca²⁺ filter and uptake Ca²⁺ ions.

In addition to being a selectivity filter, the MCU also has a luminal gate between the TMD and the CCD. This luminal gate consists of a juxtamembrane loop (JML), through which Ca²⁺ ions exit from the TMD and move to the mitochondrial matrix (41). However, this luminal gate does not appear in the four fungal MCU structures (34-37). There are two possibilities for this: the JML, which makes up this luminal gate, is so flexible that it cannot be seen on an electron density map, or the luminal gate exists only in metazoans like humans (41), not in non-metazoans like fungi. Although luminal gates are not seen in the four fungal MCUs, the presence of luminal gates means that the Ca²⁺ uptake by mitochondria through the MCUs is highly regulated.

The NTD of the MCU is also involved in controlling the Ca²⁺ uptake of the MCU (42). There is a negatively charged patch in the NTD of the MCU, which is advantageous for divalent ion binding, such as with Ca²⁺ ions. Therefore, it can be assumed that the NTD is involved in controlling the activity of the MCU by binding to Ca²⁺ ions through the negatively charged patch. The NTD of the MCU is exposed to the mitochondrial matrix, which means that the concentration of Ca²⁺ ions in the mitochondrial matrix can be sensed by means of the Ca²⁺ ions that bind to the negatively charged patch of the NTD. If the sensing by the NTD shows that the Ca²⁺ concentration in the mitochondrial matrix is too high, the activity of the MCU should be suppressed (42). To this end, it is believed that the NTD can suppress the activity of the MCU by changing the oligomerization of the MCU (42, 43).

Unlike the TMD, the NTD of fungal MCU represents the dimer of dimer, which represents the structural flexibility of the NTD (Fig. 1C) (34-37). In addition, in the MCU structure of *C. elegans*, the structure was identified in the form of NTD removal, which showed a pentameric structure unlike that of fungal MCU (38). These examples indicate that the NTD affects the oligomerization of MCUs, suggesting that the NTD may regulate the activity of MCUs by regulating their oligomerization. Although further research is needed, it seems clear that the NTD affects the activity of the MCU, which means that the regulation mechanism of the MCU, along with the regulation of the MCU by MICU1-MICU2, is very sophisticated.

EMRE, THE ESSENTIAL PIECE FOR Ca²⁺ ION PERMEATION BY THE MCU

EMRE is a small protein that consists of about 100 amino acids and is essential for the activity of metazoan MCU (31). Therefore, in order to identify the Ca²⁺ uptake mechanism of metazoan MCU at the molecular level, efforts have recently been made to identify the complex structure of the MCU and EMRE. In 2019, the structure of the human MCU and EMRE complex was identified by means of Cryo-EM, and the role of EMRE in

the MCU holo-complex uptake of Ca^{2+} ions could be identified in detail (Fig. 3A) (41).

In EMRE, the N-terminus faces the matrix of mitochondria, has a β -hairpin structure, and shows a single α -helix structure toward its C-terminus, which is embedded in the IMM (41). In the MCU and EMRE complex structure, four EMREs are bound to the tetrameric MCU, which means that the MCU and EMRE are bound at a ratio of 1:1 (Fig. 3A) (41).

The most dramatic structural feature of the MCU-EMRE complex is the dimerization of the MCU-EMRE by the interaction between the NTDs in each protomer (Fig. 3A) (41). The NTD of the MCU-EMRE induces dimerization by means of strong interaction with the NTD of another MCU-EMRE (Fig. 3A). The MCU-EMRE dimer formed in this way has a V-shape as a whole. If this structure is actually formed in the IMM, the MCU-EMRE dimer will also induce the IMM to have a V-shape (Fig. 3A). However, further research is needed to understand why EMRE changes the NTD structure of the MCU to transform the overall MCU-EMRE complex into a V-shape, and how these dramatic structural changes are related to Ca^{2+} uptake in mitochondria.

Another feature observed in the MCU-EMRE complex structure is a luminal gate that was not observed in the fungal MCU (Fig. 3B) (41). The MCU-EMRE complex structure has a β -hairpin in the N-terminus of EMRE dug into the JML direction of the MCU. This binding of EMRE causes TMH2 of the MCU and CC2 of the CCD to take place, and this structural change is thought to be accompanied by a structural change of JML constituting the luminal gate that creates an open structure for the movement of Ca^{2+} ions (Fig. 3B) (41, 44). Therefore, EMRE is essential for the activation of the MCU, because EMRE structurally

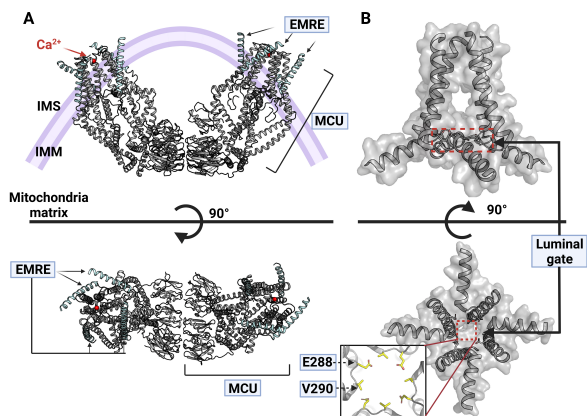


Fig. 3. Human MCU-EMRE complex structure. (A) Top and side view of hMCU-EMRE complex structure (PDB code: 6O58) (41). The hMCU is marked as a gray and the hEMRE is colored by a cyan, and the red ball is Ca^{2+} . (B) Side and bottom view of the pore region of hMCU-EMRE complex. Luminal gates are represented by red dashed box at side and bottom view. There is an enlarged figure of a luminal gate with labels of two residues (E288 and V290) which consists of luminal gate is at bottom view.

opens the luminal gate for Ca^{2+} ions to pass through.

THE MCU HOLO-COMPLEX CONTROLS Ca^{2+} UPTAKE SOPHISTICATEDLY THROUGH ITS SUBUNITS

In the IMM, the MCU holo-complex has several subunit proteins, which transport Ca^{2+} ions into the mitochondrial matrix from the IMS. MCUB, MCUR1, and MICU3 are the subunit proteins that make up the MCU holo-complex, as do MCU, EMRE, MICU1, and MICU2 (30, 45-48). However, MCUB, MCUR1, and MICU3 are not considered to be essential subunit proteins for the MCU holo-complex, because of differences in a protein function and tissue specificity for their expression (30, 45-49). In general, the essential subunit proteins that make up the MCU holo-complex are MCU, EMRE, MICU1, and MICU2 (29-31, 50-55). Therefore, in order to study the mitochondrial Ca^{2+} uptake by the MCU holo-complex and its regulatory mechanism at the molecular level, we need a MCU holo-complex structure in which the MCU, EMRE, MICU1, and MICU2 are complexed.

Recently, a MCU holo-complex with exactly such a structure has been identified (Fig. 4A) (56). MICU1 and MICU2 appear to be heterodimers in the MCU holo-complex structure that binds to the MCU-EMRE on the same side as IMS. MICU1 and MICU2 form heterodimers that are face to face and antiparallel. This heterodimer forms numerous interactions on the binding surface, indicating that these two proteins are very strongly associated (56-58). Like the previous MCU-EMRE complex structure, four molecules of the MCU and four molecules of EMRE make a complex in a 1:1 ratio in the MCU holo-complex structure. However, for MICU1 and MICU2, only one molecule of the MICU1-MICU2 heterodimer exists in the MCU holo-complex (Fig. 4A). Thus, the stoichiometry of each protein in the

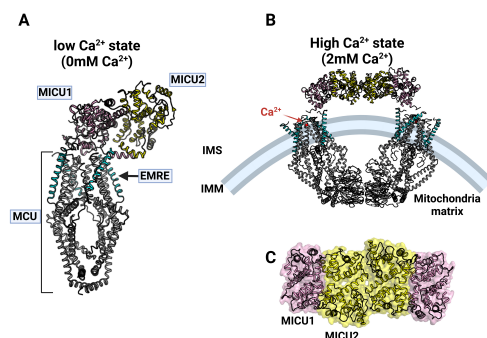


Fig. 4. Human MCU holo-complex structure. (A) Side view of hMCU holo-complex at low Ca^{2+} state (0mM experimentally) (56). MCU (gray), EMRE (cyan), MICU1 (pink) and MICU2 (yellow) are colored (PDB code: 6WDN). (B) Side view of hMCU holo-complex at high Ca^{2+} state (2 mM experimentally, PDB code: 6WDO) (56). Colors shows each component of hMCU holo-complex are described as well as (A). Ca^{2+} is represented by a red circle. (C) Dimer of MICU1-MICU2 heterodimer in hMCU holo-complex at high Ca^{2+} state.

MCU holo-complex is 4:4:1:1 (MCU:EMRE:MICU1:MICU2) (56). In the MICU1-MICU2 heterodimer, MICU1 has many interactions with MCU and EMRE, whereas in MICU2, there is only a slight bond between the C-terminus and EMRE, and little direct interaction with the MCU. Therefore, interactions between the MCU-EMRE and MICU1 play an important role in forming the MCU holo-complex (56).

The most noticeable feature of the MCU holo-complex structure shown in low Ca^{2+} concentrations is that the MICU1-MICU2 heterodimer is located above the pore part of the MCU, so that the MCU seems to be covered with a lid consisting of the MICU1-MICU2 heterodimer (Fig. 4A) (56). In this structure, MICU1 is bound to the Asp ring formed by the Asp of the DxxE motif in the MCU through ionic interactions, so that MICU1 physically makes a closed form of the MCU holo-complex. Therefore, if there are few Ca^{2+} ions in the IMS, the MCU holo-complex exhibits a closed structure and does not uptake Ca^{2+} . In addition, RuR and Ru360 compete with MICU1, which can also be confirmed in the MCU holo-complex structure of low Ca^{2+} concentrations (40). RuR and Ru360 bind to the MCU's Asp ring to prevent Ca^{2+} ions from approaching the MCU's pores, thereby inhibiting MCU activity (39). Since MICU1 also binds to the Asp ring, RuR, Ru360, and MICU1 have no choice but to compete with each other in the MCU holo-complex.

Another characteristic is that MICU1 binds in a form that blocks the entrance of the MCU when Ca^{2+} ions are few; so even if EMRE binds, the activity of the MCU holo-complex can be inhibited (Fig. 4A). EMRE is a protein that is important for the activation of metazoan MCU, and, as can be seen from the MCU-EMRE structure, the MCU can be permeable to Ca^{2+} ions, because the binding of EMRE opens the luminal gate (Fig. 3B). However, when Ca^{2+} ions are few in the IMM, even if EMRE binds to the MCU to open the luminal gate, MICU1 blocks the entrance of the MCU to inhibit access of the Ca^{2+} ions; so Ca^{2+} ions cannot pass through the MCU holo-complex. This implies that there is more than one mechanism that controls the activity of the MCU holo-complex, but also suggests that the activity of the MCU holo-complex is finely controlled by the Ca^{2+} ions present outside the MCU holo-complex by means of the MICU1-MICU2 heterodimer.

On the other hand, when Ca^{2+} concentration is high, the MCU holo-complex shows a significant structural change (Fig. 4B). When Ca^{2+} ions bind to the EF-handed motif of the MICU1-MICU2 heterodimer, the heterodimer of the MCU holo-complex interacts with the neighboring MICU1-MICU2 heterodimer, forming an O-shape overall (Fig. 4B, C) (40). The MCU-EMRE structure shown in this O-shaped MCU holo-complex structure is very similar to the V shape shown by the MCU-EMRE (Fig. 3A and 4B) although the NTD part is slightly turned (41, 56). In addition, in this structure, the luminal gate is also opened by the binding of EMRE as well as the case of low Ca^{2+} ion concentration (Fig. 4A, B). Therefore, the opening and closing of the luminal gate is entirely determined by the binding of EMRE

(41, 56).

The most distinctive feature of the O-shaped MCU holo-complex structure is that, because most of the coupling between MICU1 and the MCU disappears, MICU1 no longer covers the pore part of the MCU, and the MICU1-MICU2 heterodimer moves to edge of the MCU, thereby opening the pore completely in the MCU (Fig. 4B) (56). Accordingly, Ca^{2+} ions can access the pore of the MCU and can move to the mitochondrial matrix through the MCU. Here, the N-terminus of MICU1 interacts with the MCU and the acid C-terminus tail of EMRE binds to the basic region of MICU1, which seems to be the minimum binding needed to maintain the binding between MICU1 and MCU (56). These results allow us to understand how the MCU holo-complex in resting conditions is activated by Ca^{2+} ions and transformed into a structure that can transfer Ca^{2+} ions to the mitochondrial matrix from IMS. That is, the Ca^{2+} uptake mechanism of the mitochondria is very sophisticated and would be controlled in several stages.

DISCUSSION

In this paper, I review how Ca^{2+} ions are transported preferentially from the IMS to the mitochondrial matrix via the MCU holo-complex in mitochondria and how the activity of these MCU holo-complexes is regulated based on their protein structure. The MCU, which is the pore-forming component of the MCU holo-complex, forms an ion pore with a tetrameric structure. The DxxE motif, the motif conserved from all MCU types, configures the selective filter of the MCU such that the MCU complex can preferentially uptake Ca^{2+} ions. Through their EF-handed motif, MICU1 and MICU2 regulate the activity of the MCU holo-complex according to the concentration of Ca^{2+} ions in the IMS. Despite its diminutive size, EMRE serves as a luminal gatekeeper by means of interactions with the MCU. Simultaneously, EMRE attaches to MICU1 to mediate the binding between the MCU and the MICU1-MICU2 heterodimer in order to regulate the MCU holo-complex's ability to uptake Ca^{2+} ions based on their concentration at the IMS. Surprisingly, the binding of EMRE to the MCU causes the MCU-EMRE complex to form a V-shaped dimer, resulting in a substantial structural alteration in the MCU holo-complex.

The structure of the MCU holo-complex and each component helped us to comprehend the mechanism of Ca^{2+} uptake by the mitochondria via the MCU holo-complex in more detail. Nevertheless, there are still unanswered questions. It is unclear why the binding of EMRE generates the development of a V-shaped dimer of the MCU holo-complex and how this relates to the Ca^{2+} uptake mechanism. In addition, it also remains unclear if Ca^{2+} ions attach randomly to the MICU1-MICU2 heterodimer to activate the MCU complex or whether there is a sequential process in which the MICU1-MICU2 heterodimer interacts with Ca^{2+} ions. If we can discover answers to these questions, we will be able to comprehend not only the mechanism of Ca^{2+} uptake in mitochondria, but also the cellular Ca^{2+}

homeostasis through mitochondria.

The modulation of mitochondrial Ca^{2+} ions has lately been linked to human disorders, such as heart disease and neurological disease. It is particularly intriguing that the MCU holo-complex is intimately associated with these disorders. Therefore, a comprehension of the process of Ca^{2+} uptake in mitochondria via the structure of the MCU holo-complex will significantly aid the investigation of treatments for these disorders.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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