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Uncorking MCU to let the calcium flow

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Abstract

New cryo-electron microscopy structures of the mitochondrial Ca^{2+} uniporter ion channel complex in various conformations reveal channel gating regulation by Ca^{2+}-dependent unblock of the channel pore by MICU1.

Keywords

MCU; MICU1; EMRE; Mitochondria; Cryo-EM; Structure; Ion channel

The mitochondrial Ca^{2+} uniporter is an ion channel in the inner membrane that provides the major pathway for Ca^{2+} flux from the cytoplasm into the mitochondrial matrix. In metazoans, the MCU channel exists as a complex comprised of MCU as the tetrameric channel pore-forming subunit, EMRE, a single-pass membrane protein that is essential for channel activity, and intermembrane space (IMS)-localized MICU1/2 heterodimers that associate with the channel complex by interactions with EMRE and MCU. In a current model of MCU regulation, MICU1/2 promotes cooperative channel activation in response to Ca^{2+} binding by their EF hands, whereas apo-MICU1/2 imposes a threshold cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) below which MCU-channel activity (channel open probability P_o) is inhibited, so-called channel gatekeeping [1,2]. How MICU1/2 exerts these effects has been unknown. Furthermore, the notion of gatekeeping remains controversial [3,4]. Cryo-electron microscopy and crystallography studies have provided insights into the structures of all of the individual components, but a complete picture of the entire channel complex has been lacking. Now, a recent study published in *Nature* by Fan et al. [5] has revealed the structure of the holo-channel complex in various conformations, providing new mechanistic insights into how the channel is regulated to control Ca^{2+} fluxes into mitochondria.

Fan et al. observed the human MCU holocomplex as both monomers and V-shaped dimers of channels coupled by interactions of their matrix-localized N-terminal domains (NTD), with 4 EMRE associated with each channel, similar to that observed in the absence of MICU1/2 [6] (Fig. 1A). Ca^{2+} flux assays confirmed that the channel is functional as either a

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monomer or dimer [5, 6]. In both conformations, a single MICU1/2 heterodimer interacted with a MCU tetramer, mediated solely by MICU1, establishing the MICU1:MICU2:MCU stoichiometry in each channel as 1:1:4. In the MCU dimer complex, MICU1 interacted with MICU2 in a face-to-face configuration, whereas each MICU2 interacted with each other in a back-to-back conformation. Viewed from the plane of the membrane, the dimeric channel complex forms an “O”, with the two MCU tetramers linked in the matrix through their NTDs and in the IMS by MICU2s (Fig. 1A).

Fan et al. determined the channel structures in both Ca^{2+} and Ca^{2+}-free conditions. As expected, the MICU1/2 dimer conformations were significantly different in the two conditions. Although Ca^{2+} ions were not resolved, the conformation of MICU1/2 observed in the presence of Ca^{2+} was consistent with Ca^{2+} occupancy of all 4 functional EF hands. In this state, the MICU1/2 dimer was bound to the edge of the MCU/EMRE tetramer, with basic regions in MICU1 interacting with acidic C-termini of 2 adjacent EMREs (Fig. 1A and B). This structure suggests that EMRE likely plays an important role in tethering MICU1/2 to the channel in the presence of elevated $[Ca^{2+}]_i$, as previously suggested [7]. The structure of the selectivity filter was similar to that observed in the absence of MICU1/2, a density was observed in the selectivity filter suggesting that Ca^{2+} had access to the channel pore, and a so-called “inner gate” was not constricted, revealing no impediments to Ca^{2+} flux through the channel. Thus, the structure in the presence of Ca^{2+} is consistent with that of an open channel conformation and with previous findings that the MCU channel permits Ca^{2+} permeation in elevated $[Ca^{2+}]_i$.

A strikingly different and quite remarkable interaction of MICU1/2 with the channel was observed in the absence of Ca^{2+}. Here, MICU1 was observed to sit atop MCU, with numerous interactions in MICU1’s flat-bottomed basic N-lobe with a ring of four acidic Asp261 residues in MCU located at the entrance of the channel selectivity filter (Fig. 1C). Consequently, the apo-MICU1/2 dimer physically blocked the entrance to the pore. In this structure, the channel would be impermeable to Ca^{2+}, and therefore constitute a closed-channel conformation.

These results provide strong structural support of a model of MICU1/2-mediated channel gatekeeping and cooperative interaction, with gatekeeping now defined as channel pore block by MICU1, and cooperative activation mediated by release of block with cooperativity possibly arising from multiple Ca^{2+} binding sites in MICU1/2.

Several questions arise that include the following. Do these static structures represent those of the channel complex *in situ*? If monomers and dimers exist *in situ*, are their single-channel behaviors distinct? Whereas 4 EMRE are associated with each MCU tetramer, only two are involved in interactions with MCU in the published structures. What is the role of the other two? A recent study suggested that the MCU:EMRE stoichiometry might not be fixed, with the native complex likely containing 2 EMRE [8], a suggestion not inconsistent with the structures in Fan et al. What are the structural implications for channels containing fewer than 4 EMRE? Because of the four-fold symmetry of the Asp261 docking site in MCU, the MICU1/2 dimer can block the channel in four configurations. Whereas all four are equivalent with reference to the selectivity filter, they are distinct with reference to the

NTDs, which adopt an asymmetrical conformation. It has been suggested that MICU1/2 regulation of channel gating is influenced by matrix [Ca^{2+}], mediated at least in part by the NTDs [9]. Does the orientation of MICU1/2 binding to the holocomplex influence channel-gating regulation? How stable is the MICU1-MCU interaction in the blocked state? Is there a finite channel P_o in low-[Ca^{2+}]$_i$? Can this stability be influenced by factors in addition to Ca^{2+} binding to MICU1/2?

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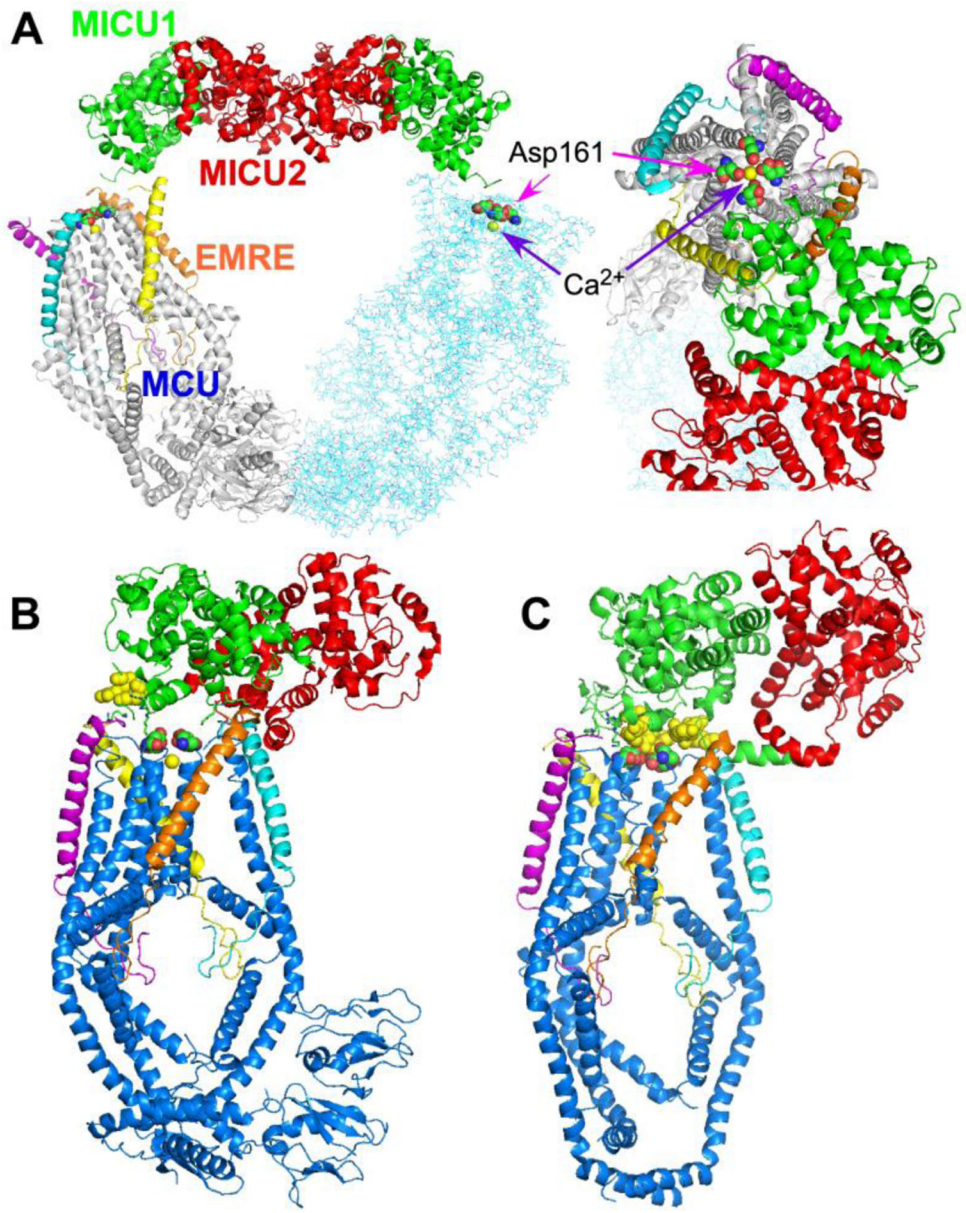

Fig. 1. MCU holocomplex structures.

(**A)** Dimeric MCU channel complex in presence of Ca^{2+}. One channel shown as cartoon, the other as lines. Left: view from plane of membrane. Right: view from intermembrane space. Ca^{2+} ion in selectivity filter shown as yellow sphere. Asp261 that provides the major docking surface for MICU1 in the closed channel conformation shown. **(B)** MCU channel complex in presence of Ca^{2+} with view rotated from (A) to reveal resolved MICU1 residues that interact with MCU in 0-Ca^{2+}, highlighted as yellow spheres. (**C)** MCU channel complex in absence of Ca^{2+} with MICU1 residues that interact with MCU Asp 261 to block entrance to the pore highlighted as yellow spheres. A and B: from pdb 6wdo; C from pdb 6wdn.