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Discussion

Outstanding questions regarding the permeation, selectivity, and regulation of the mitochondrial calcium uniporter

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ABSTRACT

The recent discovery of genes encoding the mitochondrial calcium (Ca^{2+}) uniporter has revealed new opportunities for studying how abnormal Ca^{2+} signals cause disease. Ca^{2+} transport across the mitochondrial inner membrane is highly regulated, and the uniporter is the channel that acts as a major portal for Ca^{2+} influx. Low amounts of mitochondrial Ca^{2+} can boost ATP synthesis, but excess amounts, such as following cytoplasmic Ca^{2+} overload in heart failure, triggers mitochondrial failure and cell death. In fact, precisely because mitochondrial Ca^{2+} transport is so tightly regulated, a fundamental understanding of how the uniporter functions is necessary. Two key uniporter features allow Ca^{2+} influx without mitochondrial damage during normal physiology. First, the channel is significantly more selective than other known Ca^{2+} channels. This prevents the permeation of other ions and uncoupling of the electrochemical gradient. Second, the uniporter becomes active at only high Ca^{2+} concentrations, preventing a resting leak of cytoplasmic Ca^{2+} itself. Now possessing the identities of the various proteins forming the uniporter, we can proceed with efforts to define the molecular determinants of permeation, selectivity and Ca^{2+}-regulation.

Electrogenic Ca^{2+} uptake into the mitochondria is a most remarkable evolutionary adaptation, achieved quite elegantly given the risks to the cell. Electrogenic mitochondrial uptake of any ion occurs at the expense of the gradient ($\Delta\Psi$) driving ATP synthesis, and the significant energy needed to establish it. Such a cost may be born for Ca^{2+} uptake, since in normal physiology Ca^{2+} entry can stimulate ATP production; but, when Ca^{2+} entry is excessive, it can trigger an increase in mitochondrial permeability that ultimately leads to cell death [1]. Thus, the cell has to solve a complicated problem when sequestering Ca^{2+} in mitochondria: how to admit Ca^{2+} while rejecting other cations that outnumber it by six orders of magnitude; how to do so only during signaling events; and how to restrict such influx so that mitochondria neither depolarize significantly, nor activate the permeability transition.

Of several portals for Ca^{2+} transport, the mitochondrial Ca^{2+} uniporter is the main conduit for electrogenic Ca^{2+} influx, and possesses an extremely high capacity for Ca^{2+} transport (half-saturation at ~20 mM [Ca^{2+}]) [2]. Several proteins forming subunits of this channel have been recently identified, and investigators are now poised to finally define the structural components that control mitochondrial Ca^{2+} signaling [3–10]. Here, we point out several outstanding questions regarding the permeation, selectivity, and Ca^{2+}-regulation of the uniporter pore.

A first unknown is the minimal set of protein subunits necessary to create a functional channel. To date, RNAi-mediated knockdown of several genes appears to inhibit Ca^{2+} uptake through the uniporter. Of these, the *mitochondrial calcium uniporter* (*MCU*) gene encodes the pore of the channel [3,4,11], and the *mitochondrial calcium uptake* (*MICU1*, *MICU2*, and *MICU3*) [7,8,12,13], *mitochondrial calcium uniporter* isoform *b* (*MCUb*) [10], *MCU regulator 1* (*MCUR1*) [6], *essential MCU regulator* (*EMRE*) [9], and *solute carrier 25 member 23* (*SLC25A23*) [5] genes encode proteins that appear to interact directly with *MCU*. It is unclear currently which of the proteins encoded by these regulatory genes are essential for ion flux. A proteomic analysis revealed MCUb, EMRE, and MICU proteins to be most stably associated with MCU [9], suggesting that the SLC25A23 and MCUR1 are more distant regulators that may be dispensable for ion transport. EMRE appeared to mediate the interaction between MCU and the MICU proteins. Furthermore, evolutionary analysis revealed that the *MICU* proteins co-evolved most closely with *MCU* [14], while within the particular context of mammalian systems, only elimination of *EMRE* abrogates Ca^{2+} currents to an extent comparable to *MCU* deletion [9]. Thus, it

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appears that MCU and EMRE need to be co-expressed for proper targeting or folding of the uniporter channel in mammalian systems. However, the absence of *EMRE* in lineages with *MCU* and *MICU* from plants, fungi, and protozoa suggest that in these, MCU and MICU proteins may interact directly. Finally, rare lineages possess *MCU* alone. To properly show the minimal set of proteins needed to reconstitute robust Ca^{2+} uptake, MCU variants either alone or along with EMRE and MICU will need to be purified and loaded into liposomes. To define the minimal unit forming the functional uniporter, bulk electrogenic Ca^{2+} uptake will need to be reconstituted in such purified cell-free systems.

A second unknown is how the amino acid sequence around the pore confers such extreme selectivity for Ca^{2+}. MCU has N and C termini facing the matrix, two transmembrane domains (TM1 & 2), and a short sequence that faces the mitochondrial intermembrane space connecting these two. Within this linker is a highly conserved domain (WDIMEP). The extreme Ca^{2+} selectivity of the uniporter (K_D ~2 nM) [2] is necessary to prevent mitochondrial uncoupling by the leak of other ions at resting $[Ca^{2+}]_{cyt}$. Two main structural determinants of selectivity are the pore, which permeates Ca^{2+} across the lipid bilayer, and the selectivity filter, which allows Ca^{2+} preferential access to the pore. Since MCU has two transmembrane domains, several subunits must oligomerize to form a channel. As with other multimeric ion channels, the subunits likely form a ring, with a central aqueous pore lined by one of the transmembrane domains from each MCU peptide. Since the most highly-conserved portion of the linker occurs just prior to TM2, this transmembrane domain of MCU potentially lines the aqueous pore. MCU also possesses two acidic residues in a highly-conserved region (260-Trp-Asp-Ile-Met-Glu-Pro-265, the WDIMEP motif) just N-terminal to TM2 that possesses several acidic charges, suggesting that it is involved in Ca^{2+} selectivity. Voltage-gated Ca^{2+} channels typically possess such highly-charged selectivity filters guarding the pore [15]. Furthermore, an increased density of negative residues shifts selectivity from monovalent to divalent ions, leading to preferential Ca^{2+} binding [16]. The Ca^{2+} bound at these sites then prevents permeation of monovalents. However, recent investigation of an apparently non-conductive MCU paralog (MCUb), suggests that Ca^{2+} selectivity is conferred by a more distant residue (D257V), and not the highly-conserved domain itself [10]. In the MCUb protein, which may allow Na^{+} but not Ca^{2+} permeation, the altered residue is close to TM1, and not within this DIME motif. Moreover, in voltage-gated channels, these selectivity-conferring domains sit within funnel-like pore loops, which appear absent in MCU. Their absence may suggest that the MCU selectivity filter is more constrained or rigid, given the tryptophan and proline residues in this motif, leading to high selectivity. Some evidence for such a model comes from looking at the Ca^{2+}-release activated channel, Orai. As with MCU, the selectivity filter resides in a very short domain between alpha-helical transmembrane domains, producing a high-affinity Ca^{2+} binding site (half-maximal block of monovalent current ~10 μM) [17]. In Orai this selectivity appears to be conferred by a single ring of glutamates, one from each subunit, which forms a narrow waist at the pore entrance [18]. Thus, MCU seems to possess some features similar to both these Ca^{2+} channel classes, but further definition is needed.

A third unknown is how the associated MICU subunits confer Ca^{2+}-dependent regulation of uniporter complex. Such regulation is important because the uniporter must allow Ca^{2+} entry only during signaling events, as resting transport would dissipate $\Delta\Psi$ as well. MICU1 possesses two EF hands that coordinate Ca^{2+}, is constitutively bound to MCU, and appears to face the intermembrane space [7,12,13] (though see Hoffman et. al. [19]). Such accessory subunits typically enhance channel targeting, change channel conductance, or alter its kinetics. MICU1 binding appears to enhance targeting [8] and inhibit uptake at resting $[Ca^{2+}]_{cyt}$ [12,13]. Beyond this finding, however, MICU1 function remains controversial. First, it is unclear whether the Ca^{2+}-free form of MICU1 inhibits the channel, or whether partial Ca^{2+} binding is necessary for inhibition [12,13]. Second, it is unclear if Ca^{2+} binding to MICU1 enhances uniporter activity, producing uptake greater than would be observed in the absence of MICU1 altogether. Finally, whether these effects occur through altered channel conductance or kinetics remains unknown. The proposed mechanisms for Ca^{2+} regulation have been difficult to prove due to possible confounding by secondary changes. In fact, the resting mitochondrial Ca^{2+} levels are higher after MICU1 depletion, and increased matrix Ca^{2+} buffering and efflux are also noted [12,13]. Therefore, the driving force for Ca^{2+} uptake may be reduced due to competition from buffers and efflux.

These results of future investigations into the molecular mechanisms behind mitochondrial uniporter function will be crucial to our understanding of mitochondrial Ca^{2+} physiology and how this is altered in disease, as well as necessary for novel pharmacology targeting the uniporter.

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