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The Mitochondrial Ca^{2+} Uniporter Complex

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Abstract

Calcium influx into the mitochondrial matrix plays important roles in the regulation of cell death pathways, bioenergetics and cytoplasmic Ca^{2+} signals. During the last few years, several molecular components of the inner membrane mitochondrial Ca^{2+} uniporter, the dominant pathway for Ca^{2+} influx into the mitochondrial matrix, have been identified. The uniporter is now recognized as a complex of proteins that includes a Ca^{2+} pore forming component and accessory proteins that are either required for its channel activity or regulate it under various conditions. This review summarizes recent discoveries about the molecular basis of the uniporter complex.

Introduction

Cardiomyocytes, the muscle cells comprising the majority of the structure of the heart, perform the critical physiological task of pumping blood through the heart and to the rest of the body by a process known as excitation-contraction coupling (ECC). ECC translates electrical signals originating from pacemaker cells and distributed through the heart wall by Purkinje fibers, to cardiomyocyte contraction. Whereas there are two central components of this process, excitation and contraction, the process itself has two distinct phases central to the capacity of cardiomyocytes to contract and relax repeatedly. The first phase of ECC is comprised of a series of signaling events resulting in increased intracellular calcium concentration ($[Ca^{2+}]_i$). Calcium is central to the second component of ECC, as it triggers sarcomere contraction. For cardiomyocytes to be able to relax and then contract again, Ca^{2+} must be rapidly removed from the cytoplasm. This removal is highly dependent on energy provided by ATP to drive it out of the cytoplasm against electrochemical gradients across the plasma membrane and sarcoplasmic reticulum. Cardiomyocytes must be able to vary the speed and strength at which they contract and relax in order to meet the physiological

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demands of the organism. As such, the energy available for ECC must be dynamic. At the heart of the energetic dynamism of ECC are mitochondria. Cardiomyocytes rely heavily on ATP generated from oxidative phosphorylation to match the energy demands of ECC. The role of Ca^{2+} in regulating oxidative phosphorylation and cellular bioenergetics of cardiomyocytes, has been reviewed extensively [1]. Unknown until recently have been the molecular components of mitochondrial Ca^{2+} entry. This review aims to describe the key proteins and processes that underlie mitochondrial Ca^{2+} entry, and specifically to detail the recent discoveries of the molecular constituents of the mitochondrial Ca^{2+}uniporter complex.

As summarized in many reviews and other articles in this volume and elsewhere, mitochondrial Ca^{2+} homeostasis plays important roles in cellular physiology, including cardiomyocyte functions in normal and pathophysiological conditions. In general, Ca^{2+} fluxes across the inner mitochondrial membrane (IMM) play important roles in the regulation of cell death pathways, bioenergetics and $[Ca^{2+}]_i$ signals [1-11]. The mechanisms by which mitochondrial respiration and ATP synthesis are controlled in most cells are cells are not completely understood, although respiratory control models involving kinetic feedback from the products of ATP hydrolysis, allosteric effects of ATP and P_i, rates of reducing equivalent delivery to mitochondria, O_2 availability, and various controls over respiratory chain components are involved [1]. Metabolic homeostasis in the heart during work transitions associated with varying cardiac performance has been suggested to be most critically controlled by Ca^{2+} regulation of mitochondrial bioenergetics through effects on numerous components that impinge on oxidative phosphorylation [12, 13]. The source of the mitochondrial Ca^{2+} is the cytoplasm, which in cardiac cells experiences beat-to-beat transients associated with contractions of the heart. Although there is a considerable consensus that rhythmic $[Ca^{2+}]_i$ signals drive Ca^{2+} into mitochondria, it remains controversial whether the kinetics of mitochondrial matrix $[Ca^{2+}]$ ($[Ca^{2+}]_m$) track those in the cytoplasm, or whether they are damped and effectively “average” the dynamic changes in the cytoplasm (see [14]). Mitochondria occupy over 75% of the cardiomyocyte cell volume. Nevertheless, it has been argued that mitochondrial Ca^{2+} uptake, while sufficient to maintain oxidative phosphorylation at rates sufficient to supply ATP for proper cardiac function, is nevertheless sufficiently small that it does not shape the $[Ca^{2+}]_i$ signal experienced by much of the cytoplasm, including the contractile machinery (see [15, 16]). Whether this is true within microdomains associated with Ca^{2+} release sites in close apposition to mitochondria is not as clear [17]. Furthermore, the notion that mitochondria do not shape the cardiac $[Ca^{2+}]_i$ signal is not universally accepted (see [17, 18]). The outer mitochondrial membrane is highly permeable to Ca^{2+}, primarily through the non-specific VDAC channel, whereas the Ca^{2+} permeability of the inner membrane is orders of magnitude lower and subject to significant regulation, rendering the IMM rate-limiting for Ca^{2+} influx into the mitochondrial matrix. Several specific ion channel and carrier mechanisms have been proposed to play roles in Ca^{2+} influx across cardiomyocyte IMM, which have been reviewed recently [14]. The best studied, and likely the dominant pathway is the so-called mitochondrial uniporter, which is the focus of this review. Notably, Ca^{2+} currents through the uniporter were found to be the smallest in mitochondria from cardiac myocytes compared with all other tissues examined, including skeletal muscle that had

currents 30-fold bigger [19]. Furthermore, it should be pointed out at the outset that mice with the uniporter knocked out by deletion of the pore-forming subunit (MCU, discussed below) are viable with no overt cardiac phenotypes [20]. This result is unexpected, and suggests either that cardiac myocyte mitochondria have alternate Ca^{2+} permeation pathways in the IMM or that other compensatory mechanisms can, in effect, support mitochondrial function in the absence of Ca^{2+} influx [21]. The knockout mice were viable only on a mixed genetic background [21], in agreement with the latter suggestion. Ca^{2+} influx was absent in mitochondria isolated from cardiac myocytes from the uniporter knockout mice [20], suggesting that alternative Ca^{2+} influx pathways may not compensate. However, electrophysiological analyses of these mitochondria, not undertaken, would be most informative.

Mitochondrial Ca^{2+} (Ca^{2+m}) uptake is electrogenic, driven by the large voltage present across the IMM ($\Delta\Psi_m$) developed by proton pumping by the respiratory chain [22-29]. Patch clamp electrophysiology of isolated mitoplasts, mitochondria with the outer membrane removed, demonstrated that Ca^{2+} influx was mediated by a highly Ca^{2+} selective ion channel (MiCa) [30]. A high affinity Ca^{2+} binding site (apparent $K_d < 2$ nM) endows the uniporter channel with high Ca^{2+} selectivity. Biochemical studies have suggested that the uniporter has an apparent low apparent Ca^{2+} affinity (10-70 μM) [31, 32]. However, biochemical estimates suffer to some extent from an artifact arising from the inability to voltage clamp the IMM. High Ca^{2+} permeability is most likely due to multi-ion occupancy of the pore with electrostatic repulsion between Ca^{2+} ions. The capacity of the uniporter is remarkable, with Ca^{2+} influx not saturated up to several tens of mM [30]. Of note, the uniporter channel open probability is nearly unity at normal $\Delta\Psi_m$ [30]. Under resting conditions mitochondria, including in cardiomyocytes, have $[Ca^{2+}]_m$ ~100 nM, a concentration 6 orders of magnitude lower than expected from Nernstian equilibrium considerations with normal $\Delta\Psi_m$ ~−180 mV. The high open probability and high Ca^{2+} affinity of the uniporter pore suggests that intrinsic or extrinsic regulatory mechanisms must limit its activity to prevent Ca^{2+m} overload in the face of the large thermodynamic driving force for Ca^{2+} entry. Physiological studies are consistent with an apparent low Ca^{2+} affinity of the uniporter. Agonist-induced $[Ca^{2+}]_i$ signals that reach levels comparable to those achieved during diastole in cardiomyocytes – several hundred nM - can be rapidly transduced to the mitochondrial matrix whereas similar elevations of global $[Ca^{2+}]_i$ produced by other means appear to be much less efficient [33, 34]. This has been attributed to the presence of micro-domains of high $[Ca^{2+}]_i$ that could overcome low apparent Ca^{2+} affinity of the uniporter at sites of close apposition between sites of Ca^{2+} ER/SR release and mitochondria [17, 33-40]. It has been difficult to reconcile the electrophysiological studies that suggest high apparent Ca^{2+} affinity of the uniporter with biochemical and physiological data suggesting the opposite.

The molecular identification of the uniporter was achieved only recently, and rapid developments have led to the recognition that it consists of a complex of regulatory proteins associated with a pore-forming subunit. MCU was identified by the groups of Mootha and Rizzuto as the ion-conducting pore of the uniporter [41-43, 44]. MCUb, a homolog of MCU, was identified as a dominant-negative regulator of the channel [45]. MICU1 was identified as a protein that localizes to the IMM, biochemically interacts with MCU and was required

for uniporter-mediated Ca^{2+} uptake [41, 46]. MICU2 was identified as a paralog of MICU1 that is also part of the uniporter complex [47]. MCUR1 was discovered as an IMM membrane protein with two transmembrane helices that interacted with MCU and was necessary for MCU-mediated mitochondrial Ca^{2+} uptake [48]. Expression of EMRE, which contains a single transmembrane helix, appears to mediate interactions of MICU proteins with MCU and to be required for expression of MiCa currents and MCU-mediated Ca^{2+} uptake [49].

MCU

MCU is ubiquitously expressed among organisms, including most eukaryotes with the exception of some protist and fungal branches [50]. Some MCU homologs have been detected in bacterial genomes [50]. MCU is ubiquitously expressed among human tissues. The MCU protein is predicted to have two transmembrane helices connected with a short loop containing several acidic residues, termed the DIME motif [41, 42]. Although its topology was initially debated, it has now been established that the amino- and carboxyl termini face into the matrix [51]. Thus, the short acidic loop faces into the inter-membrane space. As expected if it contributes to the uniporter pore, MCU is an oligomer, although the stoichiometry has yet to be determined. MCUb is a paralog of MCU with 50% sequence identity and similar predicted topological features. It also oligomerizes, both with itself as well as with MCU. MCUb homo-oligomers are apparently non functional since channel activity was not observed when purified MCUb protein was incorporated into planar lipid bilayers, and MCUb over-expression did not increase mitochondrial Ca^{2+} uptake [45]. MCUb can oligomerize with MCU with unknown stoichiometry i*n vivo* and in expression studies, where it may exert a dominant negative effect on MCU since co-expression reduced the number of channels observed in bilayer recordings, and over-expression reduced mitochondrial Ca^{2+} uptake [45]. MCUb was found in a complex with stably-expressed epitope-tagged MCU in HEK-293 cells [49], suggesting that it might form part of the channel pore *in vivo*. Notably, MCU and MCUb have distinct expression profiles [45]. Thus, the relative expression of the two MCU proteins may help establish the magnitude of the uniporter Ca^{2+} permeability. Of interest, MCUb mRNA is three-fold greater than MCU mRNA in heart [45], which may account at least in part for the relatively small MiCa currents observed in cardiac mitoplasts [19].

That MCU is the pore forming domain of the uniporter is supported by several lines of evidence. First, it was shown that its purification and reconstitution into planar lipid membranes of recombinant human MCU generated single channel Ca^{2+} currents with conductance at negative voltages ~ 7 pS that were blocked by Gd^{3+} and ruthenium red (RuR) and activated by strongly hyperpolarized voltages [42]. These properties are consistent with that of the uniporter current. Mutation of two acidic residues in the DIME motif resulted in no observable channel activities. These mutations also abolish uniporter mediated mitochondrial Ca^{2+} uptake [41, 42]. The conductance and gating features of the single channel currents were nevertheless significantly different from those recorded from the endogenous uniporter by patch clamp electrophysiology of isolated mitoplasts from Cos-7 cells [30]. In particular, the so-called MiCa currents had a smaller single channel conductance and prolonged openings at hyperpolarized voltages [30] that were not observed

in the reconstituted channels. To more strongly establish that MCU was the pore forming subunit, a mutant MCU was recorded by patch clamp electrophysiology of mitoplasts. Replacement of a serine residue in the DIME motif with alanine greatly reduced the RuR sensitivity of mitochondrial Ca^{2+} uptake [41]. Expression of S259A MCU conferred reduced RuR sensitivity of uniporter currents in patch-clamped mitoplasts, in a dominant negative manner, strongly implicating MCU as the pore forming subunit [43]. More recently, expression of *Dictyostelium* MCU was sufficient to confer mitochondrial RuR-sensitive mitochondrial Ca^{2+} uptake in yeast that lack all uniporter components [44]. In contrast, *Dictyostelium* MCU with the DIME motif acidic residues mutated was inactive. Together, these results indicated that MCU is the Ca^{2+} pore.

MICU1

The discovery of MICU1 by Perocchi et al. [46] heralded in the molecular age of the mitochondrial uniporter. It was discovered in a cleverly conceived targeted RNA interference screen as a protein whose expression was required for MCU-mediated Ca^{2+} uptake [46]. Subsequent studies confirmed that knockdown of MICU1 inhibits mitochondrial Ca^{2+} uptake [52, 53]. However, Mallilankaraman et al. ([54]) found that reduction of MICU1 expression lead to constitutive Ca^{2+m} overload as a consequence of enhanced uniporter activity under resting conditions [54]. In that study, MICU1 appeared to function to limit MCU-mediated Ca^{2+} uptake at low $[Ca^{2+}]_i$ (< 3 μM). In contrast, MICU1 appeared to be without effect on uniporter-mediated Ca^{2+} uptake at higher $[Ca^{2+}]_i$. MICU1 has two Ca^{2+} binding EF hands [55]. Mutation of key residues to disrupt Ca^{2+} binding in either EF hand inhibited the ability of MICU1 to exert its inhibitory function. It was therefore suggested that the EF hands provide a high-affinity $[Ca^{2+}]$ sensing mechanism that enables MICU1 to exert its regulation when $[Ca^{2+}]_i$ is at resting levels (<100 nM). It was concluded that MICU1 provides a “gatekeeping“ function to minimize uniporter activity under resting conditions, protecting mitochondria from chronic Ca^{2+} overload. This mechanism, rather than intrinsic low Ca^{2+} affinity of MCU, accounts for the apparent low Ca^{2+-} affinity of the uniporter [56].

Subsequent studies have confirmed that mitochondria in cells with MICU1 knocked down gain the ability take up Ca^{2+} at low $[Ca^{2+}]_i$ (< ~2-3 μM) [57-59]. Whether lack of MICU1-associated “gatekeeping” results in mitochondrial Ca^{2+} overload may depend on several variables, including cell type, number and activity of uniporter channels in each mitochondrion, other regulatory mechanisms, etc. Thus, it is perhaps not surprising that constitutive mitochondrial Ca^{2+} loading in cells with MICU1 knocked down was observed in some studies [54, 59] but not in others [46, 57, 58]. Particularly revealing however, have been studies in cells from human patients in which loss of function mutations in *MICU1* cause brain and muscle disorders [60]. $[Ca^{2+}]_m$ was found to be constitutively elevated in cells from these patients, and agonist-induced mitochondrial Ca^{2+} uptake was not inhibited [60]. Discrepant results in different studies regarding the effects of MICU1 on mitochondrial Ca^{2+} uptake under resting- and agonist–stimulated conditions might be due to compensatory mechanisms, for example changed mitochondrial Ca^{2+} buffering capacity [57] that operated in some studies but not in others.

Importantly, more recent studies have demonstrated that altered expression of components of the uniporter complex can influence the protein levels of the other components, in a cell type specific manner [53]. Thus, the interpretations of experiments based on changing MICU1 expression are complicated, and the mechanisms by which MICU1 regulates MCU activity have become unclear and controversial. There is a debate regarding the role of cytoplasmic Mg^{2+} in the gatekeeper function of MICU1 [54, 57]. There is disagreement about whether MICU1 also has roles in regulating uniporter activity at $[Ca^{2+}]_i > 3$ μM (the high $[Ca^{2+}]$ regime) [53, 54, 57-59]. There is disagreement about the roles of the EF hands. In the studies of Mallilankaraman et al. [54], mutation of the EF hands phenocopied knockdown of MICU1, suggesting that the EF hands are Ca^{2+}-liganded under resting conditions with high affinity that mediates inhibition of MCU activity under basal conditions. In agreement, Hoffman et al. [61] reported that MiCa currents were elevated in cells with MICU1 knocked down as well as in cells expressing MICU1 with both EF hands mutated. In contrast, Csordás et al. [57] and Kamer and Mootha [53] found that MICU1 with non-functional EF hands was still capable of inhibiting Ca^{2+} uptake. Whether MICU is a membrane protein is also unclear. MICU1 was originally shown to be associated with the IMM [46]. It is predicted to have a transmembrane domain and it is relatively resistant to carbonate extraction [57], suggesting that it might be an integral membrane protein. Fluorescence recovery after photobleaching (FRAP) revealed expressed MICU1 to have a higher mobility than that of MCU, although the mobile fraction was only 40% [61]. The location of the EF hands is also debated. Plasma membrane Ca^{2+} channels are associated with cytoplasmic–localized intrinsic or extrinsic mechanisms that sense Ca^{2+} that has fluxed through the channel to provide, generally, negative feedback regulation. In agreement, the Madesh group concluded in their original study [54] as well as in a subsequent one using distinct approaches [61] that MICU1 senses $[Ca^{2+}]_m$. In contrast, several groups have concluded that the EF hands are localized in the inter-membrane space [53, 55, 57, 59, 62].

Crystal structures of human MICU1 revealed MICU1 dimers. Each monomer contains an N-domain (residues 103-177), N-lobe (183-318), C-lobe (324-445) and a C-helix (445-465) in a structure encompassing residues 103 to the carboxyl terminus at position 476. To obtain MICU1 crystals in the presence of Ca^{2+}, the protein was truncated by deletion of the C-helix. Comparison of the dimeric structures in the presence and absence of Ca^{2+} revealed, first, that MICU1 bound 2 Ca^{2+} in canonical EF hands, one each in the N- and C-lobes. Second, that two pseudo-EF hands are present that do not bind Ca^{2+}. Third, that whereas the overall topology of the monomers was similar in the presence and absence of Ca^{2+}, Ca^{2+} binding caused large conformational changes in the EF hands that resulted in the formation of a new dimer interface associated with a large rotation of one monomer with respect to the other. Biochemical studies had suggested that conserved cysteines may play a role in MICU1-2 dimerization [59]. In contrast, they do not appear to be involved in homoligomerization in the MICU1 crystal structure. In the crystal structures of MICU1 solved in the absence of Ca^{2+}, the carboxyl-terminus was packed into the center of a trimer-of-dimers hexameric structure [55]. Surprisingly, the apo-MICU1 was also largely present as a hexamer in solution. In contrast, other biochemical studies indicate that MICU1 homo-oligomerizes as a dimer [55, 59, 61] with no evidence for higher order structures. Nevertheless, the C-helix may have functional significance. MICU1 with the C-helix deleted

failed to immunoprecipitate MCU, and cells expressing the truncated MICU1 were severely deficient in agonist-induced mitochondrial Ca^{2+} uptake [55], suggesting that the C-elix plays an important functional role that cannot be discerned from the crystal structures. Similarly, expression of MICU1 lacking the carboxyl-terminal 31 amino acids distal to the second EF hand abolished its ability to rescue the loss of gatekeeping function in the low [Ca^{2+}] regime [53]. Deletion of an amino-terminal polybasic domain cripples the ability of MICU1 to regulate C-MCU [61]. This region is present only in part in the amino-terminal part of the N-domain. The structure provides no insights into the role of the polybasic region in MCU regulation.

MICU2

MICU2 has 27% sequence identity with MICU1. It also contains two putative EF hands and is localized in mitochondria. Proteinase sensitivity assays suggested an inter-membrane space localization [59], similar to the localization of MICU1 using this assay. Importantly, MICU2 interacts with MICU1, and furthermore its protein expression levels depend upon MICU1 expression [47, 53, 59]. Whether changes in MICU2 expression affect MICU1 expression is controversial [47, 53, 59]. MICU2 resides in a complex with MICU1 and MCU [47, 53, 59] dependent upon MICU1 and EMRE [53, 59] (discussed in more detail below). MICU1and MICU2 appear to physically interact *in vivo* [59]. It has been suggested that MICU1-MICU2 dimers predominate *in vivo* [59]., A model has been proposed in which MCU binds EMRE, which binds dimeric MICU1-MICU2 through MICU1 [53].

MICU2 expression is required for MCU function since either its knockout or mutation of its EF-hands inhibited mitochondrial Ca^{2+} uptake in response to a high [Ca^{2+}] pulse [47, 53]. MICU2 also appears to be required for the gatekeeping function that had been attributed to MICU1 [53]. The effect of knocking down MICU2 expression were smaller than those observed by knocking down MICU1 [47, 53]. It has been suggested that MICU1 function does not require MICU2, but that the converse is not true [53]. Interestingly, EF-hand mutant MICU1 could rescue the gatekeeping function that was compromised in cells lacking MICU2, whereas wild-type MICU1 could not [53].

Patron et al. [59] noted that MICU1 over-expression or knock-down both enhanced the $[Ca^{2+}]_m$ response to strong agonist stimulation. Potentiation by MICU1 of mitochondrial Ca^{2+} uptake in the high [Ca^{2+}] regime required functional EF hands, suggesting that they are in the apo-form in resting conditions, in contrast to the conclusions reached by Mallilankaraman et al. [54] in their original description of MICU1 as a gatekeeper in the low [Ca^{2+} regime]. In the Patron study [59] MICU2 knock-down also enhanced the $[Ca^{2+}]_m$ response to strong agonist stimulation, in contrast to results from the Mootha group [47, 53]. In the Patron study, MICU2 overexpression, unlike MICU1 overexpression, did not enhance mitochondrial Ca^{2+} uptake in the high [Ca^{2+}] regime, suggesting non-redundant functions [59]. These results were interpreted to suggest that MICU1 is an activator of MCU, whereas MICU2 is the gatekeeper, by the following reasoning. Overexpression of MICU1 generates MICU1-homodimers in lieu of MICU1-2 heterodimers, relieving MICU2-mediated inhibition, providing additional stimulation and accounting for enhanced mitochondrial Ca^{2+} uptake in the high $[Ca^{2+}]_i$ regime. In the low [Ca^{2+}] regime, the absence of MICU2 in

complex with MICU1 in MICU1-overexpressing cells abolishes the gatekeeping function conferred by MICU2. This accounts for elevated agonist-induced $[Ca^{2+}]_m$ in MICU1 knockdown-as well as overexpressing-mitochondria [59]. However, it is important to note that mitochondrial Ca^{2+} uptake was not examined in the low $[Ca^{2+}]$ regime where gatekeeping is functional. Thus, the claim that MICU2 rather than MICU1 is the uniporter gatekeeper has not yet been convincingly established. Nevertheless, electrophysiological experiments provide support for the scheme proposed by Patron et al. [59]. Addition of purified MICU1 protein to planar lipid bilayers containing MCU failed to enhance channel activities until $[Ca^{2+}]$ was raised from nil to 1 μM, suggesting that MICU1 is a MCU activator. In contrast, addition of purified MICU2 protein in 0-Ca^{2+} completely inhibited MCU channel activity, suggesting that MICU2 is a channel inhibitor in the low $[Ca^{2+}]$ regime.

MCUR1

MCU Regulator 1 (MCUR1) was discovered in an RNAi screen of mitochondrial genes that regulate mitochondrial Ca^{2+} uptake [48]. It is a ~40KD mitochondrial inner membrane protein with two transmembrane spanning helices, with amino- and carboxyl-termini facing into the inter-membrane space with a loop connecting the helices present in the matrix. Knockdown of MCUR1 abolished uniporter activity. MCUR1 interacted with MCU, but not with MICU1. MCUR1 has a wide tissue distribution, including the heart [48]. MCUR1 was not detected in a proteomics study of the uniporter complex associated with immunoprecipitated epitope-tagged MCU in HEK-293 cells (below) [49]. Thus, additional studies are required to establish the mechanisms and role of MCUR1 in the uniporter complex.

EMRE

Essential MCU Regulator (EMRE) was discovered by mass spectrometry analysis of the MCU proteome [49]. It is a 10 kD protein with a single predicted transmembrane domain with a highly acidic carboxyl-terminus. It is widely expressed among mammalian tissues, and its protein stability is dependent upon the presence of MCU. Its knockout abolished uniporter-mediated Ca^{2+} uptake and strongly reduces MiCa currents. Furthermore, the interaction of MICU1 and 2 with MCU is abolished in EMRE depleted cells, although their expression is unchanged. Thus, EMRE expression is required for uniporter activity even in the presence of the other known uniporter components, and it is required to mediate the interaction of MICU1/2 with MCU [49]. Nevertheless, MICU1 and MICU2 can be co-immunoprecipitated in the absence of EMRE [49]. The molecular details of its topology, and interactions with MCU and MICU proteins are not yet known. A polybasic region in the MICU1 amino-terminus was found to be important in the biochemical interaction with MCU [61]. Thus, whether EMRE mediates all of the interactions between MICU proteins and MCU remains to be determined. Of interest, EMRE expression is absent in some organisms that express MCU, including *Dictyostelium discoideum*. Nevertheless, *Dictyostelium* uniporter activity is present, and recombinant *Dictyostelium* MCU expressed in MCU knockout mammalian cells can rescue mitochondrial Ca^{2+} uptake [44]. Of note, expression of *Dictyostelium* MCU did not restore expression of EMRE, and *Dictyostelium* MCU was

functional in EMRE knockout cells, indicating that in mammalian cells, as in *Dictyostelium*, *Dictyostelium* MCU can function in the absence of EMRE, in contrast to mammalian MCU [44]. Yeast lack MCU and other components of the uniporter complex. Reconstitution of *Dictyostelium* MCU confers mitochondrial Ca^{2+} uptake in yeast. In contrast, human MCU was ineffective in this regard unless it was reconstituted with human EMRE [44].

Conclusions

Here we have described the molecular components of the mitochondrial Ca^{2+} uniporter, a key pathway that enables cytoplasmic Ca^{2+} signals to be coupled to bioenergetic regulation necessary for proper cardiac function. With this collection of components in hand, it is now imperative to clarify their roles, stoichiometries and regulation in uniporter function. There is as yet no coherent model of the uniporter complex and how it functions. Although technically demanding, electrophysiology of the uniporter, combined with mutagenesis and expression manipulation, will provide more detailed insights that cannot be obtained by other approaches that necessarily infer the activity and regulation of the channel by its various components. Protein biochemistry and structural determinations of the uniporter components and of their complexes are largely lacking but are necessary to provide insights and bases for testing hypotheses about how the specific components interact in various conditions. Analyses of post-translational modifications of uniporter components that affect the function of the uniporter channel will likely yield important insights into cell specific roles and functionality of the uniporter. Along these lines, it is of interest that the activity of the cardiac uniporter has been suggested to be regulated by MCU phosphorylation [63, 64] under physiological and pathological conditions, although this has become controversial [65, 66]. Of considerable interest is the lack of expected overt cardiac phenotypes in a mouse with MCU genetically deleted [20]. New animal models, together with the discoveries of other important genes that contribute to uniporter function promise to provide a wealth of new insights into the roles of the mitochondrial Ca^{2+} uniporter in cardiac physiology during the next several years.

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Highlights

- Calcium influx into the mitochondrial matrix plays important roles.
- Molecular components of the mitochondrial Ca^{2+} uniporter have been identified.
- The uniporter includes a Ca^{2+} pore forming component and accessory proteins.
- We review the functional and molecular properties of the uniporter complex.