The molecular era of the mitochondrial calcium uniporter

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Abstract | The mitochondrial calcium uniporter is an evolutionarily conserved calcium channel, and its biophysical properties and relevance to cell death, bioenergetics and signalling have been investigated for decades. However, the genes encoding this channel have only recently been discovered, opening up a new 'molecular era' in the study of its biology. We now know that the uniporter is not a single protein but rather a macromolecular complex consisting of pore-forming and regulatory subunits. We review recent studies that harnessed the power of molecular biology and genetics to characterize the mechanism of action of the uniporter, its evolution and its contribution to physiology and human disease.

Mitoplast
A mitochondrion without the outer membrane.

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Calcium is an incredibly versatile signalling ion that has been linked to diverse processes from fertilization and muscle contraction to cell death. It has been aptly dubbed the 'life and death signal' (REF. 1). Mitochondria are among the most important regulators and targets of calcium signalling; these organelles can be positioned strategically throughout the cell and have established roles in energy homeostasis and even cell death.

Evidence for a close relationship between calcium and mitochondria dates back more than 50 years, when several groups simultaneously discovered that purified and energized mitochondria take up and buffer large amounts of calcium when provided with phosphate[2–4]. It is notable that much progress was made in understanding mitochondrial calcium physiology at the same time that the principles of chemiosmotic coupling were being elucidated. Later studies showed that calcium transport across the mitochondrial inner membrane occurs through a 'uniporter', which transports calcium in an electrogenic manner, thereby dissipating the membrane potential generated by the respiratory chain, without requiring co-transport with an anion or exchange for another cation[4]. Flux studies in isolated mitochondria suggested the existence of a channel mechanism[4], which was validated by mitoplast electrophysiology studies that confirmed the remarkably high conductance and selectivity of the uniporter[5]. Studies carried out in parallel showed that the primary mechanism for calcium efflux from mitochondria is electroneutral exchange with sodium[6] or hydrogen[7] (FIG. 1a).

Since these early studies, many roles have been ascribed to the uniporter. For example, calcium in the mitochondrial matrix was shown to activate three matrix dehydrogenases[8]. Consistent with this observation, uniporter-mediated calcium uptake can lead to an alteration in the redox metabolic state of mitochondria, so that following a transient alteration in cytosolic calcium concentration, mitochondria retain a 'metabolic memory' of the event[9]. In addition, mitochondrial uptake of calcium can shape the frequency and amplitude of cytosolic calcium waves[10]. Finally, almost all forms of cell death are associated with overload of calcium in mitochondria[11,12].

Although great progress was made in understanding the physiology of the uniporter, its molecular identity remained a mystery. Most early studies monitored the uptake of calcium into mitochondria using either small molecule or genetic reporters of calcium and were aided by the use of ruthenium red and Ru360 as highly potent chemical inhibitors of the uniporter. These drugs have been particularly useful for studies of purified mitochondria and for electrophysiology studies of mitoplasts[5]. However, they are not cell permeable and have additional targets within the cell[13,14], which limits their use *in vivo* and in intact cells.

A series of discoveries during the past five years, propelled by advances in genomics, has identified the genes encoding the uniporter. We now appreciate that it is a macromolecular complex consisting of pore-forming and regulatory subunits. With the molecular identities of the uniporter proteins established, we can now delve deeper into how the complex works, and why it is important in cells and in whole organisms. Here, we review recent progress in our understanding of the uniporter mechanism and its role in physiology and disease, and outline pressing unanswered questions that should drive progress in the field in the coming years.

EF hand
A calcium-binding motif consisting of a helix–loop–helix structure.

Molecular components of the uniporter complex

For decades, the molecular identities of the proteins that constitute the uniporter remained unknown. The completion of the mammalian mitochondrial protein inventory MitoCarta marked the first step towards the molecular era of study of the uniporter[15]. Classical physiological observations were also crucial: it was shown more than four decades ago that *Saccharomyces cerevisiae* lack mitochondrial uniporter activity[16], whereas trypanosome mitochondria show membrane potential-dependent calcium uptake into mitochondria in a ruthenium red-sensitive manner, which is compatible with uniporter activity[17]. A search for MitoCarta proteins that have homologues in trypanosomes but not in yeast led to the identification in 2010 of mitochondrial calcium uptake protein 1 (MICU1), which was the first uniporter component to be reported[18]. MICU1 is a calcium-binding protein containing an EF hand domain that is resident in the mitochondrial intermembrane space and has been proposed to be a regulatory subunit. The pore-forming subunit mitochondrial calcium uniporter protein (MCU) was identified soon after by an integrative genomics approach using MICU1 and the MitoCarta inventory[19,20]. MICU2 and MCU regulatory subunit b (MCUb; also known as CCDC109B), which are paralogues of MICU1 and MCU, respectively, were subsequently confirmed to be involved in the uniporter complex[21,22]. Both MICU2 and MCUb physically interact with other uniporter components and seem to regulate channel activity. Finally, in 2013, the uniporter holocomplex (also termed the uniplex) was characterized for the first time, using affinity purification and quantitative proteomics, which showed that the complex also contains an additional small membrane-spanning protein, essential MCU regulator (EMRE; also known as SMDT1)[23]. EMRE was shown to be an essential member of the uniporter complex and to be required for mitochondrial calcium uptake[23].

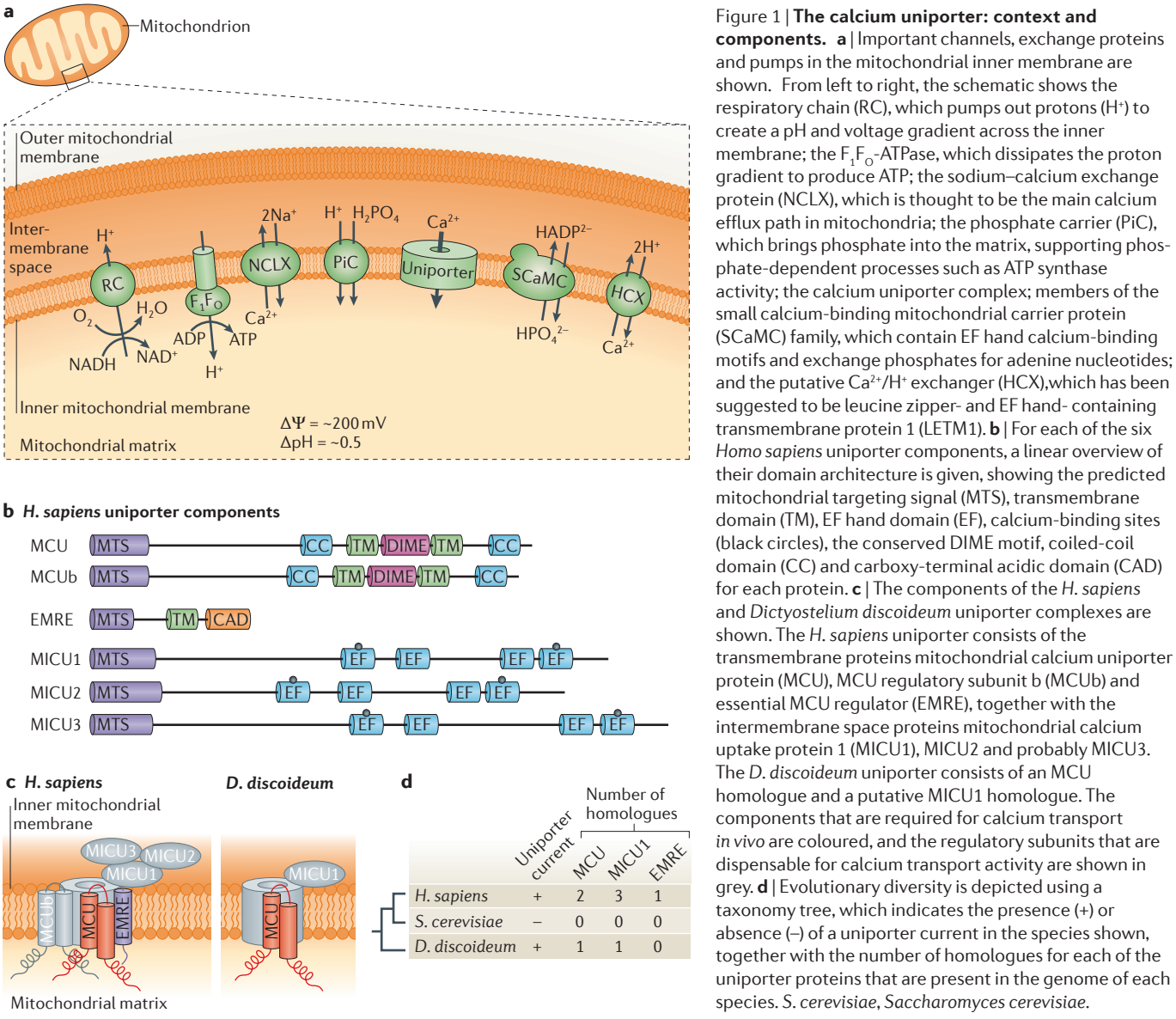

Figure 1 | **The calcium uniporter: context and components.** **a** | Important channels, exchange proteins and pumps in the mitochondrial inner membrane are shown. From left to right, the schematic shows the respiratory chain (RC), which pumps out protons (H^+) to create a pH and voltage gradient across the inner membrane; the F_1F_O-ATPase, which dissipates the proton gradient to produce ATP; the sodium–calcium exchange protein (NCLX), which is thought to be the main calcium efflux path in mitochondria; the phosphate carrier (PiC), which brings phosphate into the matrix, supporting phosphate-dependent processes such as ATP synthase activity; the calcium uniporter complex; members of the small calcium-binding mitochondrial carrier protein (SCaMC) family, which contain EF hand calcium-binding motifs and exchange phosphates for adenine nucleotides; and the putative Ca^{2+}/H^+ exchanger (HCX),which has been suggested to be leucine zipper- and EF hand- containing transmembrane protein 1 (LETM1). **b** | For each of the six *Homo sapiens* uniporter components, a linear overview of their domain architecture is given, showing the predicted mitochondrial targeting signal (MTS), transmembrane domain (TM), EF hand domain (EF), calcium-binding sites (black circles), the conserved DIME motif, coiled-coil domain (CC) and carboxy-terminal acidic domain (CAD) for each protein. **c** | The components of the *H. sapiens* and *Dictyostelium discoideum* uniporter complexes are shown. The *H. sapiens* uniporter consists of the transmembrane proteins mitochondrial calcium uniporter protein (MCU), MCU regulatory subunit b (MCUb) and essential MCU regulator (EMRE), together with the intermembrane space proteins mitochondrial calcium uptake protein 1 (MICU1), MICU2 and probably MICU3. The *D. discoideum* uniporter consists of an MCU homologue and a putative MICU1 homologue. The components that are required for calcium transport *in vivo* are coloured, and the regulatory subunits that are dispensable for calcium transport activity are shown in grey. **d** | Evolutionary diversity is depicted using a taxonomy tree, which indicates the presence (+) or absence (–) of a uniporter current in the species shown, together with the number of homologues for each of the uniporter proteins that are present in the genome of each species. *S. cerevisiae*, *Saccharomyces cerevisiae*.

Although this study only considered a single cell type (HEK-293T cells), all of these components — MCU, MCUb, MICU1, MICU2 and EMRE (FIG. 1b,c) — seem to be ubiquitously expressed in all mammalian tissues. MICU3, which is a paralogue of MICU1 and MICU2, is preferentially expressed in the central nervous system (CNS), raising the possibility that there is tissue-specific variation in the composition of the uniporter complex[21].

The minimal uniporter

Since the initial discovery of MCU, several lines of evidence have indicated that it is essential for calcium transport and that it is the pore-forming subunit of the uniporter complex. In the initial reports that identified MCU, it was shown that MCU oligomerizes[19], and that calcium uptake activity is abolished by mutations affecting key acidic residues (including D261A and E264A)[19,20] that may interact with calcium ions and provide a selectivity filter by analogy to other calcium channels[24]. A single point mutation (S259A) of MCU close to the predicted entrance to the channel confers almost complete resistance to the classical uniporter inhibitor Ru360 (REF. 19), which suggests that MCU is either the direct target of Ru360 or is mechanistically very close to the target. Importantly, voltage-clamping experiments in whole mitoplasts later confirmed that loss of MCU indeed leads to a loss of the electrophysiologically defined uniporter current[25]. Furthermore, the MCU homologue in *Dictyostelium discoideum* is by itself sufficient to reconstitute mitochondrial calcium transport when expressed in yeast, which lacks uniporter components altogether[26] (FIG. 1c,d).

Although there is broad consensus that human MCU is indeed the pore-forming subunit, it has not been clear whether it is sufficient for reconstitution of the channel. *In vitro* experiments initially suggested that human MCU alone is sufficient to transport calcium in planar lipid bilayers[20]; however, the electrophysiological properties of this transport were distinct from those that were previously reported for the uniporter[25]. Moreover, expression of human MCU alone in yeast mitochondria does not reconstitute uniporter activity[26]. This discrepancy was resolved by the discovery of EMRE, which is a metazoa-specific protein that is part of the uniporter complex and is necessary for uniporter activity in mammalian cells[23]. Heterologous co-expression of EMRE and human MCU is sufficient to reconstitute uniporter activity in yeast[26]. It thus seems that the minimal human uniporter *in vivo* requires both MCU and EMRE (FIG. 1c). Furthermore, the observation that the *D. discoideum* MCU homologue conducts calcium in the absence of an EMRE homologue is consistent with the molecular phylogeny, as EMRE homologues only occur in metazoa[23] (FIG. 1d). These findings together definitively show that MCU is involved in forming the pore of the channel; however, they also raise the question of how metazoan MCU differs from MCU homologues in other organisms that lack EMRE. Interestingly, EMRE in mammalian cells seems to have two prominent functions: it is required for uniporter current and it mediates the interaction of MICU1 and MICU2 with MCU[23].

Regulation of the uniporter by MICU1 and MICU2

Mitochondria can discriminate between different types of incoming calcium signal[1]. It has long been known that the uniporter is activated by cytosolic calcium[27–29], although the underlying molecular mechanisms were not clear. The calcium-binding EF hand-containing proteins MICU1 and MICU2, which associate with the pore complex in the intermembrane space[23,30–32], now seem to be key mediators of uniporter signal processing.

When MICU1 was first identified, loss-of-function studies resulted in a severe defect in mitochondrial calcium handling, which established MICU1 as a key regulator of the uniporter[18]. However, its precise function was not clear. Subsequent studies clarified that MICU1 functions as a 'gatekeeper' that sets the threshold of extramitochondrial calcium concentration for uptake of calcium into mitochondria[30,33]. Since then, MICU2, which is a paralogue of MICU1, has been found to be closely linked to MICU1 both physically and functionally[21]. In fact, knockout of either *MICU1* or *MICU2* in cells results in mitochondria taking up calcium at a lower threshold of calcium concentration[34].

Understanding the roles of MICU1 and MICU2 — and, in particular, the differences between them — has been challenging (reviewed in REF. 35). Initially, there was uncertainty regarding the submitochondrial localization of MICU1 and MICU2, although orthogonal methods now clearly indicate that they are localized to the intermembrane space[23,30–32]. In addition, the cross-stabilization of uniporter proteins, which is especially prominent between MICU1 and MICU2 (REFS 21,34), has complicated the interpretation of knockdown experiments. Moreover, silencing of these components can also decrease the expression levels of MCU in a tissue-specific and cell type-specific manner, which makes it difficult to determine whether a phenotype is directly or secondarily related to the gene that is being silenced. However, with the advent of new knockout technologies, including transcription activator-like effector nucleases (TALENs)[36] and CRISPR[37], it is now possible to address these issues. For example, complete knockout of MICU2 enables MICU1 to be manipulated independently of MICU2.

It is now becoming clear how MICU1 and MICU2 work together to sense cytosolic calcium concentration and to influence the activity of the pore[34,38] (FIG. 2). The emerging model is that the combination of MICU1 and MICU2 regulates the uniporter to prevent calcium at low concentration from passing through, and that MICU2 has an inhibitory role[34,38]. However, the role of MICU1 is debated: in one model, MICU1 is thought to tonically inhibit the uniporter[34], and a rise in cytosolic calcium concentration disinhibits the pore activity; another model proposes that a rise in calcium concentration has a solely activating effect on MCU through MICU1 (REF. 38). Another important difference between the two models relates to the independence of MICU2: in the first model, MICU2 works together (in series) with MICU1, and the presence of MICU1 is required for inhibition of the channel; by contrast, in

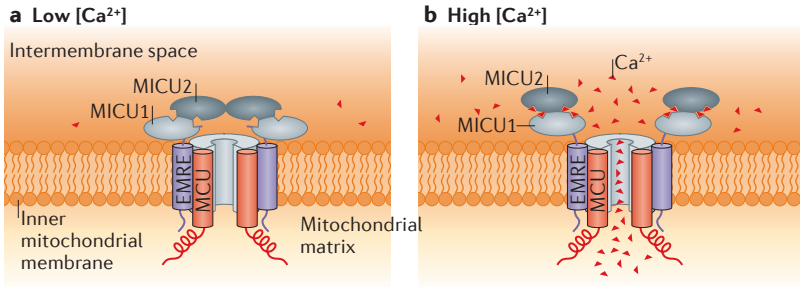

Figure 2 | **Regulation of the uniporter by calcium.** The current model for the regulation of uniporter activity by calcium is shown in schematic form. **a** | When calcium concentration ([Ca^{2+}]) in the intermembrane space is low, mitochondrial calcium uptake protein 1 (MICU1) and MICU2 inhibit calcium uptake through the uniporter. **b** | When [Ca^{2+}] rises, such as during a signalling event, the inhibition is relieved and calcium is transported through the uniporter. The red triangles indicate calcium ions. As the mechanism by which MCUb regulates uniporter activity is currently unclear and MCUb is dispensable for uniporter activity, and MICU3 is largely expressed in the central nervous system, these proteins have not been depicted in this model.

the second model, MICU2 can have an inhibitory function completely independent of MICU1. Additional studies will be required to clarify the specific roles of MICU1 and MICU2.

The crystal structure of MICU1 has recently been reported[39] and it confirms that MICU1 has two calcium-binding EF hand domains, each of which is paired with a structural EF hand that does not bind to calcium. An amino-terminal truncation of MICU1 forms a trimer of dimers in the apo (unbound) state, whereas the calcium-bound form (with an additional carboxy-terminal truncation) forms a dimer, but may form higher order oligomers when the C-terminus is present. However, several important questions remain. For example, both the oligomeric state of MICU1 *in vivo* and how MICU1 interacts with the rest of the MCU-containing complex, including MICU2, are not clear. MICU1 and MICU2 may interact through a mixed disulfide bond formed by cysteine residues at their C-terminal ends[38], although evidence for direct binding is still lacking. However, this putative disulfide bond is dispensable for the cross-stabilization of MICU1 and MICU2 *in vivo*, as removing the whole C-terminal helix of MICU1 does not inhibit the stabilization of MICU2 by MICU1 (REF. 34). Additional structural information, in combination with careful genetic studies, will be crucial to understanding the regulatory logic of MICU1 and MICU2.

Additional layers of regulation

Molecular studies show that several mechanisms, in addition to those involving MICU1 and MICU2, are likely to be involved in the regulation of uniporter activity. It has long been known that uniporter activity can vary between developmental time points[40] and between tissues[41]. This temporal and tissue-specific control seems to be mediated by a combination of regulatory modes.

Several additional proteins within the uniporter complex might tune uniporter activity. MICU1 and MICU2 have another paralogue in mammals: MICU3, the expression of which is largely confined to the CNS. Although this has not yet been studied, if MICU3 functions similarly to MICU1 and MICU2, it may be an important CNS-specific regulator of incoming calcium signals. It has been suggested that MCUb is a direct negative regulator of the uniporter complex[22]. MCUb, similarly to its paralogue MCU, has two predicted transmembrane domains (FIG. 1b). It has been shown to interact with MCU by co-immunoprecipitation[22,23] and to reduce mitochondrial calcium uptake[22]. The mechanism by which MCUb regulates uniporter activity is still unclear; however, learning more about calcium transport through the pore and about how MCUb is associated with MCU may help to shed light on MCUb-mediated regulation.

Other mitochondrial inner membrane proteins have been proposed to regulate uniporter activity. However, it is unclear whether their roles are direct (that is, acting on the uniporter complex itself) or indirect (such as affecting the driving force for calcium uptake). For example, genetic manipulation of small calcium-binding mitochondrial carrier protein 3 (SCaMC3; also known as SLC25A23) has shown that this protein is important for uniporter activity; however, SCaMC3 has long been known to control phosphate transport[42], which is itself crucial for uniporter activity, as phosphate buffers calcium within the matrix. In addition, RNAi-mediated knockdown of coiled-coil domain-containing 90A (*CCDC90A*; also known as *MCUR1*) alters mitochondrial calcium uptake. In one study, CCDC90A was shown to co-immunoprecipitate with MCU[43], although another study characterizing MCU-interacting proteins did not identify CCDC90A[23]. However, CCDC90A has an orthologue in yeast (which lacks uniporter activity) that was recently shown to be important for the assembly of cytochrome *c* oxidase (complex IV)[44]. Taken together, in our opinion, the data provide strong evidence that the involvement of CCDC90A in uniporter activity is indirect, probably through effects on the respiratory chain, which generates the driving potential for mitochondrial calcium transport. Other proteins, including the mitochondrial sodium–calcium exchange protein NCLX (also known as SLC8B1)[45], the leucine zipper- and EF hand-containing transmembrane protein 1 (LETM1)[7], and mitochondrial uncoupling protein 2 (UCP2) and UCP3 (REF. 46), have been shown to influence mitochondrial calcium uptake and efflux, although this is likely to occur through other mechanisms that are independent of the uniporter complex.

New studies are beginning to elucidate how the uniporter is regulated at the level of gene expression. Transcriptional regulation has been shown to occur through the calcium-dependent transcription factor cAMP response-element binding protein, which binds to the *MCU* promoter[47]. Synaptic activity was shown to repress transcription of *Mcu* through a calmodulin kinase-dependent mechanism[48]. Post-transcriptional regulation has been shown in some cell lines to involve microRNA-25 (miR-25), which can decrease *MCU* gene expression and activity[49]. In the coming years, it will be exciting to see how direct activity of the uniporter may be able to feed back into these gene regulatory networks to influence its own expression.

The uniporter in physiology and disease

Before the molecular discovery of the uniporter machinery, the hypothesized physiological roles of the uniporter, which were based on correlative studies, included feed-forward control of ATP homeostasis through calcium-mediated activation of matrix dehydrogenases, modulation of the duration of cytosolic calcium signals by buffering cytosolic calcium, and control of cell death. The identification of the molecular components of the uniporter provides the opportunity to test these and other hypotheses more directly using genetic tools (TABLE 1).

Cellular studies. The uniporter seems to be dispensable in cell culture, as complete loss of MCU is compatible with the growth and proliferation of cultured cells at baseline[23,50]. Conveniently, this makes it possible to revisit the three hypothesized roles of the uniporter using cell culture as a model system.

Several studies now support a role for the uniporter in what we term excitation–energetic coupling through the activation of matrix dehydrogenases. Both the activity of the pyruvate dehydrogenase (PDH) complex and ATP concentration can be affected by the manipulation of components of the uniporter *in vitro*. PDH is activated indirectly by mitochondrial matrix calcium through the calcium-sensitive PDH phosphatase catalytic subunit 1 (PDPC1; also known as PDP1): calcium activates PDPC1, which dephosphorylates PDH, thereby increasing its activity[8,50]. Uniporter activity should thus be correlated with decreased phosphorylation of PDH and increased PDH activity. As expected, loss of MCU in mitochondria from skeletal muscle results in increased phosphorylation of PDH and concomitantly decreased PDH activity[50]. *MICU1* knockdown in HeLa cells, which leads to increased basal levels of calcium in the mitochondrial matrix, decreases the phosphorylation of PDH as expected[33]. Furthermore, pancreatic β-cells with ablated MCU have decreased ATP concentration following glucose stimulation. This contributes to diminished glucose-stimulated insulin secretion[51], which seems to be particularly important for sustained insulin secretion[52]. Thus, several *in vitro* studies provide compelling genetic evidence that the uniporter has a role in excitation–energetic coupling.

Genetic manipulation of MCU has also revealed the involvement of the uniporter in regulating transient fluxes of cytosolic calcium, which is a process that seems to have many links to immune function. For example, it was shown that calcium signalling downstream of the leukotriene receptor is influenced by the uniporter[53]. Knocking down *Mcu* in rat basophils resulted not only in ablated mitochondrial calcium uptake in response to stimulation of the leukotriene receptor with leukotriene C4 (LTC4) but also in faster dampening of cytosolic calcium oscillations and suppression of calcium-dependent gene expression following stimulation. The uniporter seems to be important for two additional processes that are relevant to immune signalling: store-operated calcium entry (SOCE) and activation of the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) complex termed the NLRP3 inflammasome. Specifically, loss of MCU has been shown to reduce SOCE after inositol trisphosphate-mediated calcium release[54] and to blunt activation of the NLRP3 inflammasome induced by both the membrane attack complex in human lung epithelial cells[55] and by *Pseudomonas aeruginosa* in airway epithelial cells from patients with cystic fibrosis[56]. Taken together, these lines of evidence confirm a role for the uniporter in cellular calcium signalling and point to its importance in the immune system.

The role of the uniporter in cell death remains ambiguous and controversial: genetic manipulation of uniporter components has yielded evidence both supporting and refuting the involvement of the uniporter in cell death. In support of a role for uniporter function in exacerbating apoptosis, it has been shown that *MICU1*-knockdown human cell lines and MCU-overexpressing human cell lines — both of which have increased uniporter activity — are sensitized to apoptotic stress[20,33]. Similarly, MCU overexpression in *Trypanosoma brucei* leads to increased sensitivity to apoptotic stress[57]. Consistent with these results, increased levels of miR-25 (which lead to decreased MCU levels) are protective against apoptotic stimuli, whereas anti-miR-25 (which leads to increased MCU levels) sensitizes cells to apoptotic stimuli[49]. However, several other studies found no difference in sensitivity to apoptosis with genetic manipulation of MCU. These studies include MCU overexpression in a human breast adenocarcinoma cell line[58] and *Mcu*-knockout mouse embryonic fibroblasts[50] exposed to apoptotic stimuli. Further studies will be required to understand the precise role of the uniporter in cell death.

Whole organism studies. Perhaps the greatest surprise in the molecular era of the uniporter is that whole organisms can survive without the uniporter. *Mcu* knockout can be tolerated by many organisms, including trypanosomes, worms and mice. Although cellular studies clearly indicate that genetic manipulation of the uniporter can affect processes such as neurotransmission, growth and differentiation, and basic immune function, whole organisms are tolerant of uniporter loss. Here, we discuss the consequences of complete loss of MCU *in vivo*.

Loss of MCU in *T. brucei* has several consequences *in vivo*[57]. Both the procyclic and bloodstream trypanosome forms have a growth defect as a result of *MCU* knockdown. In the procyclic form, loss of MCU results in increased autophagy, which is consistent with experiments in HeLa cells[33]. It is of particular interest that loss of MCU in the bloodstream trypanosome form has consequences, including growth-rate defects, even though these trypanosomes have almost no mitochondrial oxidative phosphorylation and rely on glycolysis for survival. These data indicate that the uniporter has roles in addition to activating the mitochondrial matrix dehydrogenases to increase flux through the mitochondrial respiratory chain. Perhaps most remarkably, loss of MCU in these bloodstream forms of *T. brucei* results in decreased infectivity in mice. The mechanism for this effect requires further investigation, which may lead to a better understanding of the involvement of mitochondrial calcium in infectivity and could suggest a therapeutic target for trypanosomal disease.

Excitation–energetic coupling
The coupling of cellular ATP consumption with its production.

Leukotriene receptor
A receptor that is present in immune cells, which can be activated by leukotrienes to lead to an inflammatory cascade.

Store-operated calcium entry
(SOCE). A mechanism to replenish endoplasmic reticulum calcium stores, which occurs through calcium release-activated channels in the plasma membrane.

NLRP3 inflammasome
A large protein complex that is part of the innate immune system; it can be activated by many different stimuli to trigger inflammatory processes.

Table 1 | **Functions of the uniporter that have been identified by genetic manipulation of uniporter complex components**

Process	Genetic perturbation	Observation	Correlation of uniporter activity to process	Refs
Autophagy	MCU KD	Increased autophagy in HeLa cells and the procyclic form of *T. brucei*	Negative	33, 57
	MCU KO	No increased autophagy in mouse heart and liver or in MEFs	None	50
Cell death	MICU1 KD	Increased sensitivity of HeLa cells to apoptotic stimuli	Positive	33
	MCU overexpression	Increased sensitivity to apoptotic stimuli in HeLa cells or *T. brucei*, but not human breast cancer MDA-MB-231 cells	Positive or none	20, 57, 58
	miR-25 overexpression	Protects HeLa cells against apoptotic stimuli	Positive	49
	Anti-miR-25 expression	Sensitizes human prostate cancer PC3 cells and human colon cancer HCT116 cells to apoptotic stimuli	Positive	49
	MCU KD	Inhibits celastrol-induced paraptosis in human breast cancer MDA-MB-435S cells	Positive	68
	MCU KO	No differences in response of MEFs to apoptotic or necrotic stimuli	None	50
Generation of mtROS	MCU KD	No increased mtROS in MDA-MB-231 breast cancer cells	None	58
	MICU1 KD	No increased mtROS in MDA-MB-231 breast cancer cells	None	58
	MICU1 KD	Increased basal mtROS in HeLa and endothelial cells	Positive	33
	MICU1 KD	Increased mtROS during SOCE but not at baseline in HeLa cells	Positive	30
	MCU overexpression	Increased mtROS in *T. brucei*	Positive	57
Oncogene-induced senescence	MCU KD	Escape from replicative and oncogene-induced senescence in human mammary epithelial cells	Positive	69
	MICU1 KD	Sensitizes human mammary epithelial cells to oncogene-induced senescence	Positive	69
Matrix dehydrogenase activation and metabolic coupling	MCU KO	Mouse skeletal muscle has decreased PDH activity and increased serum lactate levels after starvation	Positive	50
	MICU1 KD	Decreased levels of PDH phosphorylation in HeLa cells	Positive	33
	MCU KD	Increased starvation-induced phosphorylation of AMPKα (which is sensitive to the AMP:ATP ratio) in MDA-MB-231 cells	Positive	58
Leukotriene receptor signalling	MCU KD	Decreased LTC4-induced oscillations in cytosolic calcium concentration and resulting calcium-dependent gene expression in rat basophilic leukaemia RBL-1 cells	Positive	53
Cell migration	MICU1 KD	Impaired endothelial cell migration	Negative	33
	MICU1 overexpression	Increased cell migration in CVD endothelial cells	Negative	70
Neuron excitotoxicity	MCU overexpression	Increased basal toxicity and vulnerability to NMDA receptor-dependent excitotoxicity in mouse neurons	Positive	48
	MCU KD	Protection against NMDA receptor-dependent excitotoxicity in mouse neurons	Positive	48
Inflammasome activation	MCU KD	Decreased membrane attack complex-induced NLRP3 inflammasome activation in human lung epithelial cells	Positive	55
	MCU KD	Decreased *P. aeruginosa*-induced NLRP3 inflammasome activation in cystic fibrosis human airway epithelial cells	Positive	56
Glucose-stimulated insulin secretion	MCU KD	Attenuated glucose-induced insulin secretion in pancreatic β-cells	Positive	51, 52
Cardiomyocyte contraction	MCU KD	Increased contraction in neonatal cardiomyocytes.	Negative	71
SOCE	MCU KD	Impaired SOCE in HeLa cells following $InsP_3$-mediated calcium release.	Positive	54
T. brucei pathogenesis	MCU KD/KO	Decreased viability of bloodstream and procyclic forms	Positive	57
	MCU KD	Decreased infectivity to mice	Positive	57

Table 1 (cont) | **Functions of the uniporter that have been identified by genetic manipulation of uniporter complex components**

Process	Genetic perturbation	Observation	Correlation of uniporter activity to process	Refs
C. elegans wound healing	MCU KO	Defective wound closure, which may be mediated by mtROS	Positive	59
D. rerio development	MCU KD	Alterations during gastrulation, including blastomere convergence and extension movements	Positive	65
M. musculus exercise tolerance	MCU KO	Decreased skeletal muscle performance	Positive	50
M. musculus skeletal muscle trophism	MCU overexpression	Leads to skeletal muscle hypertrophy	Positive	72
	MCU KD	Leads to skeletal muscle atrophy	Positive	72
M. musculus heart rate increase	DN-MCU expression	Ablated catecholamine-induced heart rate increase with expression in cardiac pacemaker cells	Positive	61
H. sapiens disease	MICU1 deletion	Encephalopathy, muscle myopathy, learning disability and movement disorder.	Positive	62

AMPKα, AMP-activated protein kinase-α; *C. elegans*, *Caenorhabditis elegans*; CVD, cardiovascular disease; *D. rerio*, *Danio rerio*; DN-MCU, dominant-negative MCU; *H. sapiens*, *Homo sapiens*; $InsP_3$, inositol trisphosphate; KD, knockdown; KO, knockout; LTC4, leukotriene C4; *M. musculus*, *Mus musculus*; MCU, mitochondrial calcium uniporter protein; MEFs, mouse embryonic fibroblasts; MICU1, mitochondrial calcium uptake protein 1; miR-25, microRNA-25; mtROS, mitochondrial reactive oxygen species; NLRP3, NOD-, LRR- and pyrin domain-containing 3; NMDA, *N*-methyl-D-aspartate; *P. aeruginosa*, *Pseudomonas aeruginosa*; PDH, pyruvate dehydrogenase; SOCE, store-operated calcium entry; *T. brucei*, *Trypanosoma brucei*.

More recently, the orthologue of MCU in *Caenorhabditis elegans* was knocked out, leading to ablated mitochondrial calcium uptake as anticipated[59]. These worms are both viable and fertile. The phenotype observed was a deficiency in the wound-healing process, which is proposed to involve uniporter-mediated control of the production of mitochondrial reactive oxygen species (mtROS). However, cellular studies have produced conflicting results as to whether MCU regulates the generation of mtROS[33,57,30,58].

Loss of MCU can also be tolerated in mice with a mixed genetic background[50]. *Mcu* knockout was found to be lethal for C57BL/6 mice, whereas knockout mice with an outbred CD1 background were viable, albeit with reduced numbers[60]. *Mcu*-knockout CD1 mice were overtly normal but had reduced exercise tolerance. This could be consistent with a cellular role for the uniporter in stimulating activity of the tricarboxylic acid (TCA) cycle during calcium signalling events (such as muscle contraction during exercise). In addition to whole-body *Mcu* knockout, loss of uniporter activity in sinoatrial node cells in mice through the overexpression of a dominant-negative MCU protein also suggests a link between uniporter activity and cellular energetics[61]. In this case, although wild-type and mutant animals were indistinguishable at base line, the blunted increase in heart rate of mutant mice in response to β-adrenergic stimuli revealed a role for uniporter activity in the 'fight-or-flight' response. Finally, knockdown or overexpression of *Mcu* in mouse skeletal muscle recently revealed a relationship between uniporter activity and skeletal muscle trophism, which is probably mediated by the transcriptional co-activator PPARγ co-activator 1α, isoform 4 (PGC1α4): decreased *Mcu* expression resulted in skeletal muscle atrophy, and increased *Mcu* expression resulted in hypertrophy. Overall, mouse studies are beginning to reveal the physiological importance of the mammalian uniporter, including in skeletal muscle and in response to adrenergic stimuli.

Human disease. Inborn errors of the uniporter in humans are starting to be recognized. Truncating mutations in *MICU1* that lead to complete loss of its expression result in a Mendelian syndrome of skeletal muscle myopathy, learning disability and movement disorder[62]. The pathology resulting from loss of MICU1 manifests in a tissue-specific manner, which is reminiscent of other mitochondrial disorders[63]. However, loss of MICU1 does not coincide with impairment of the activity of the respiratory chain. It is notable that MCU loss in mice and MICU1 loss in humans both result primarily in skeletal muscle pathology. An important future challenge lies in determining whether patients with mutations in *MICU1* have pathological involvement of other organ systems.

Future directions

The molecular era of uniporter study is still in its infancy. We have an exciting opportunity now to link decades of biochemical and physiological studies to their molecular basis. Although there has been remarkable progress in the past five years, some very basic information is still lacking, and there is much left to learn.

At present, we do not know the stoichiometry and oligomeric state of each component of the uniporter complex, or whether the stoichiometry is static or dynamic. Investigating how the composition of the uniporter complex might vary between cell types and developmental stages will be crucial. Quantitative proteomics to identify the components of the complex has only been carried out so far in HEK-293T cells. It is already clear that the relative expression levels of uniporter components vary depending on tissue type[21], and it has been shown in whole-mitoplast patch-clamp experiments that the uniporter current similarly varies between tissues[41]. These observations raise the possibility that there are other components of the uniporter complex in other types of cell or tissue. In fact, we have hypothesized that MICU3 is likely to be an important

Tricarboxylic acid (TCA) cycle
A series of enzymatic reactions in the mitochondrial matrix that take acetyl coenzyme A through a series of oxidation steps, which are important for many biosynthetic pathways and also produce reducing equivalents to feed into the respiratory chain. Two enzymes in the TCA cycle, α-ketoglutarate dehydrogenase and isocitrate dehydrogenase, are activated by matrix calcium ions.

Skeletal muscle myopathy
A disorder of skeletal muscle that can manifest as weakness, cramps or exercise intolerance.

regulator of the uniporter in the CNS[21], although this protein has yet to be studied in detail.

High-resolution structures will be crucial for understanding the activity and regulation of the uniporter channel. On the basis of the few but high-quality electrophysiology studies that have been reported, we know that the uniporter has remarkably high conductance and selectivity for calcium. How does the uniporter overcome the trade-off between conductance rate and selectivity? Structures of each component of the complex may provide insight into both the calcium-transport properties and the signal processing of the uniporter. In particular, it will be important to learn how MICU1 and MICU2 relay information to the pore, and whether MCUb can form heterooligomers with MCU.

The molecular discovery of the uniporter machinery has uncovered several evolutionary paradoxes. In a seminal study, Carafoli and Lehninger showed that although vertebrate mitochondria have uniporter activity, yeast mitochondria do not[16]. This finding was crucial to the discovery of the uniporter machinery and in fact, as predicted, yeast do not have MCU or MICU1 homologues[64]. However, the same paper showed that *Neurospora crassa* also does not have classical uniporter activity, although we now find that this organism has an MCU homologue[64]. What is the function of the *N. crassa* MCU homologue? Does it conduct calcium, perhaps with different kinetic or pharmacological properties? Has it evolved to transport a different ion? Another paradox involves EMRE: in mammalian cells, it seems that the interaction of MICU1 and MICU2 with MCU is mediated by EMRE. However, EMRE seems to have emerged in metazoa, so how does MICU1 relay regulatory information to the MCU pore in species such as trypanosomes that lack an EMRE homologue? There is certainly a rich source of information waiting to be tapped from studies of the evolutionary biology of the uniporter.

Phylogenetic analysis indicates that MCU and MICU1 were present in the earliest mitochondria — but if the uniporter is so evolutionarily ancient, how can its complete loss be tolerated in whole organisms? We note that in mice, there does seem to be a selective advantage for animals with a functional uniporter, as the animals lacking uniporter activity do not breed in the anticipated Mendelian ratios[60]. In addition, zebrafish with *mcu* knocked down have developmental defects in gastrulation[65]. After birth, however, the main difference reported between whole-body *Mcu*-knockout mice and wild-type mice is an intolerance to exercise in the absence of uniporter activity. Thus, it seems likely that as for other proteins involved in bioenergetics, such as creatine kinase[66] and myoglobin[67], the presence of a functional uniporter confers a fitness advantage[50] that may be subtle in laboratory studies but is highly selected for over evolutionary timescales. Given the growing appreciation of a role for the uniporter in immune signalling (TABLE 1), it is conceivable that the uniporter complex confers a fitness advantage in response to pathogens that has favoured its retention.

The field has long suspected that the mitochondrial calcium uniporter and its biology would be relevant to human disease. The molecular discovery of the uniporter now makes it possible to evaluate its causal role in human disease. Already, we have seen the first reports of human patients with mutations in *MICU1*, who present with neuromuscular disease. In the coming years, with advances in next-generation sequencing, we anticipate that other disorders — both rare and common — will be linked to the uniporter, raising the prospect that targeting this complex may have therapeutic benefits.

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Competing interests statement
The authors declare no competing interests.