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TOPICAL REVIEW

The mitochondrial calcium uniporter in the heart: energetics and beyond

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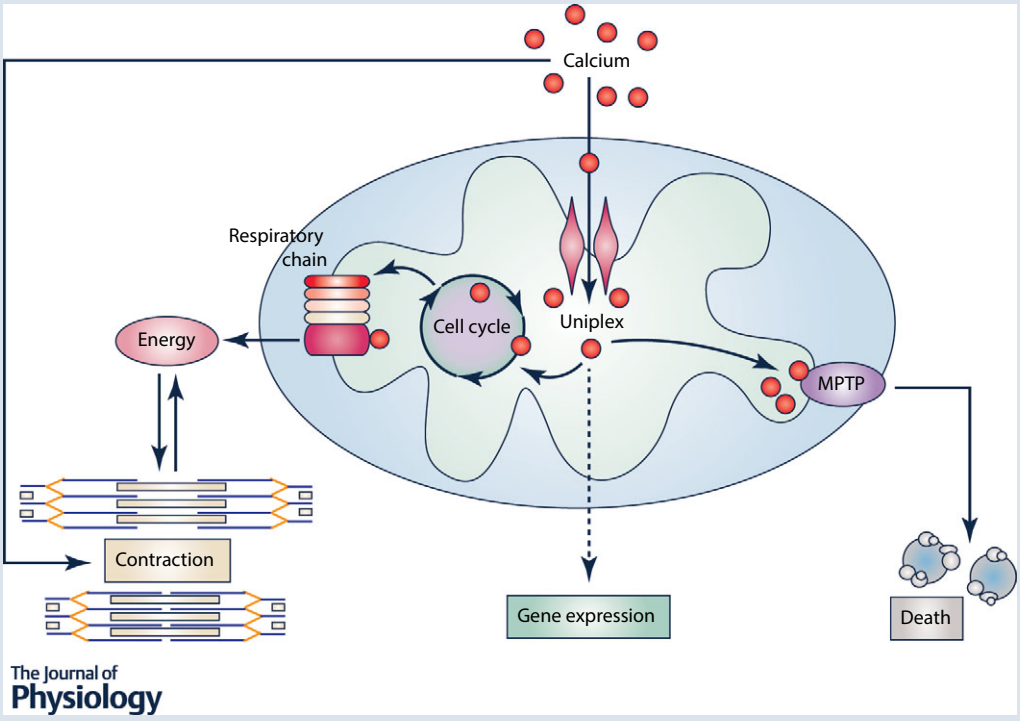

Abstract Ca^{2+} and mitochondria are inextricably linked to cardiac function and dysfunction. Ca^{2+} is central to cardiac excitation–contraction coupling and stimulates mitochondrial energy production to fuel contraction. Under pathological conditions of dysregulated Ca^{2+} cycling, mitochondrial Ca^{2+} overload activates cellular death pathways. Thus, in the cardiomyocyte, the mitochondrial Ca^{2+} microdomain is where contraction, energy and death collide. A key component of mitochondrial Ca^{2+} signalling is the mitochondrial Ca^{2+} uniporter complex (uniplex), an inner membrane Ca^{2+} transporter and major pathway of mitochondrial Ca^{2+} entry. Once known only as the unidentified target for ruthenium red and related compounds, in recent

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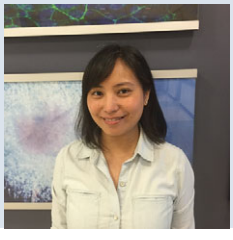

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years, the uniplex has evolved into a complex multiprotein assembly. The identification of the molecular constituents of the uniplex has made possible the generation of targeted genetic models to interrogate uniplex function *in vivo*. This review will summarize our current understanding of the molecular structure of the uniplex, its impact on mitochondrial energetics and cardiac physiology, its contribution to cardiomyocyte death, and its expanding roles in cardiac biology.

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Abstract figure legend The mitochondrial calcium uniporter complex (uniplex) is a major mode of mitochondrial Ca^{2+} import. Mitochondrial Ca^{2+} signalling and the uniplex have pleiotropic functions in the heart. The uniplex-dependent mitochondrial Ca^{2+} signal links the energy demands of cardiac contraction to mitochondrial energy supply. Under pathological conditions of cytosolic Ca^{2+} overload as experienced in heart failure, uniplex-dependent transport of Ca^{2+} into the mitochondria can trigger mitochondrial permeability transition pore (MPTP) opening and activation of death pathways. Finally, the uniplex's function may extend beyond the mitochondrion to impact global cellular gene expression.

Abbreviations EMRE, essential MCU regulator; MICU1, mitochondrial calcium uptake 1; MICU2, mitochondrial calcium uptake 2; MCU, mitochondrial calcium uniporter; MCUb, mitochondrial calcium uniporter b; MCUR1, mitochondrial calcium uniporter regulator 1; MPTP, mitochondrial permeability transition pore complex; ROS, reactive oxygen species; RyR1, ryanodine receptor; TRPC3, transient receptor potential canonical 3; SLC25a23, solute carrier family 25 member 23.

Introduction

Ca^{2+} is a ubiquitous intracellular second messenger with pleiotropic functions ranging from controlling gene expression to regulating cardiac contraction, activating metabolism and initiating cell death (Orrenius & Nicotera, 1994; van Haasteren *et al.* 1999; Bers, 2008; Balaban, 2009). It has long been recognized that at the mitochondria, Ca^{2+} signalling can drive both cellular metabolism and death. Under physiological conditions, mitochondrial Ca^{2+} serves as a signal to enhance energy production by activating three dehydrogenases of the tricarboxylic acid (TCA) cycle (pyruvate dehydrogenase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase) (Denton, 2009), as well as the ATP synthase (Jouaville *et al.* 1999) (Fig. 1). Under pathological conditions of cytosolic Ca^{2+} overload, however, Ca^{2+} signalling at the mitochondria, instead of upregulating metabolism, actually engages mitochondrial death pathways. Mitochondrial Ca^{2+} overload triggers the opening of the mitochondrial permeability transition pore (MPTP), leading to permeabilization of the mitochondrial inner membrane, mitochondrial dysfunction and cell death (Haworth & Hunter, 1979; Hunter & Haworth, 1979; Kwong & Molkentin, 2015) (Fig. 2). In addition to its role relating to energy and the permeability transition, mitochondrial Ca^{2+} plays an important part in regulating the cellular redox state. Ca^{2+} activation of TCA cycle dehydrogenases controls NADH production, which in turn impacts cellular anti-oxidative capacity regeneration and mitochondrial reactive oxygen species (ROS) production (Kohlhaas *et al.* 2010). Thus, Ca^{2+} signalling at the mitochondria converges on a number of cellular life and death pathways.

Ca^{2+} and mitochondria have special relevance to the heart, as Ca^{2+} is at the core of cardiac excitation–contraction coupling (Bers, 2002), and mitochondria contribute largely to both cardiac physiology and pathophysiology. The heart is heavily reliant on mitochondria as a source of ATP. Indeed, mitochondria constitute ∼30% of the cardiomyocyte's volume and supply > 90% of the ATP required for cardiac contraction (Harris & Das, 1991; Piquereau *et al.* 2013). Given that Ca^{2+} underlies both contraction and enhancement of mitochondrial ATP production, mitochondrial Ca^{2+} microdomains – sites of high local cytosolic Ca^{2+} established by the close apposition of mitochondria to regions of Ca^{2+} release, such as the sarcoplasmic reticulum, which facilitate mitochondrial Ca^{2+} transport – are a critical integration point where ATP supply can be coordinated to meet the energetic demands of contraction (Balaban, 2009). In the face of pathological levels of Ca^{2+}, however, mitochondria serve as a platform whereby death pathways are engaged, and the mitochondrial Ca^{2+} overload–MPTP axis of cell death has been shown to contribute largely to the cardiomyocyte death observed following cardiac ischaemia–reperfusion injury (Griffiths & Halestrap, 1995; Murphy & Steenbergen, 2008) (Fig. 2). Moreover, during heart failure, alterations in cytosolic Na^+ and Ca^{2+} handling impair mitochondrial Ca^{2+} influx, thereby potentiating bioenergetic mismatch and aberrant ROS production, which may collectively secondarily engage the MPTP and contribute to adverse cardiac

remodelling and the progression to heart failure (Kohlhaas *et al.* 2010; more comprehensively reviewed in this issue by Maack *et al.*). Thus, the mitochondrial Ca^{2+} microdomain presents an intriguing target to control cardiac function through modulating energetics and death.

It has long been appreciated that mitochondrial Ca^{2+} influx can be inhibited by the drugs ruthenium red and its derivative Ru360 (Zazueta *et al.* 1999). Indeed, ruthenium red and Ru360 have been demonstrated to inhibit Ca^{2+} overload-induced cell death in numerous models including neuronal excitotoxicity (Dessi *et al.* 1995) and cardiac ischaemia–reperfusion injury (Garcia-Rivas *et al.* 2006; Zhang *et al.* 2006), underscoring the power of modulating mitochondrial Ca^{2+} handling. The molecular identification of the target of ruthenium red and Ru360, the mitochondrial calcium uniporter (MCU), has generated intense interest in the study of the mitochondrial Ca^{2+} dynamics (Baughman *et al.* 2011; De Stefani *et al.* 2011). MCU is the core component of the multi-protein MCU holocomplex (uniplex), an inner membrane Ca^{2+} transporter and a major mode of mitochondrial Ca^{2+} entry (Kamer & Mootha, 2015). This discovery has paved the way for the genetic manipulation of mitochondrial Ca^{2+} influx and identification of molecular regulators, and importantly, has set the stage for the study of mitochondrial Ca^{2+} signalling *in vivo*.

This review will focus on MCU and the uniplex in the regulation of cardiac function and dysfunction. We will (1) summarize the current understanding of the molecular architecture of the uniplex, (2) review lessons learned from genetically modified mice on MCU's contribution to cardiac energetics and cardiac physiology, (3) define the contribution of MCU to the regulation of cardiac pathology, and (4) explore new pathways that MCU signalling may impact.

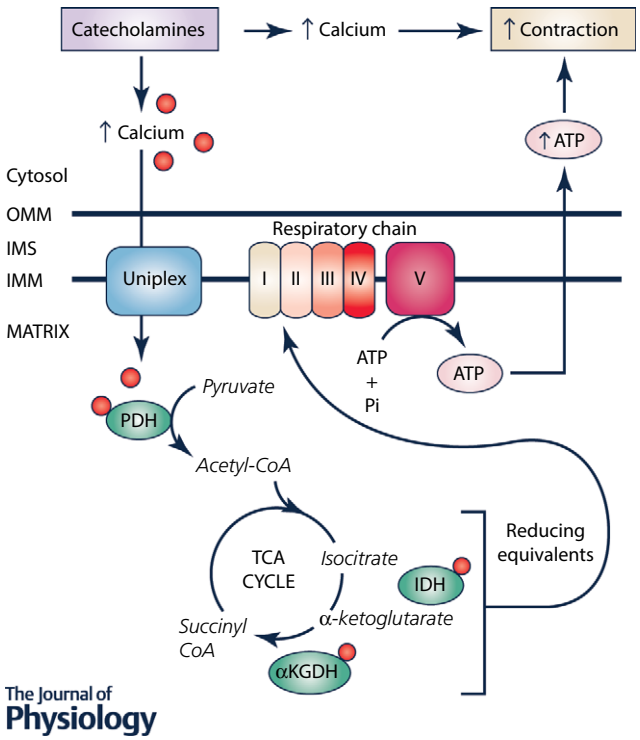

Figure 1. Mitochondrial Ca^{2+} signalling in cardiac metabolic contraction coupling
In the cardiac fight or flight response, catecholamine signalling leads to increased cytosolic Ca^{2+} that drives enhanced contraction. This enhanced contraction is coupled to elevated mitochondrial energetic output as the uniplex transports Ca^{2+} into the mitochondrial matrix. Ca^{2+} activates the TCA cycle enzymes pyruvate dehydrogenase (PDH), isocitrate dehydrogenase (IDH), and α-ketoglutarate dehydrogenase (αKGDH), as well as the mitochondrial ATP synthase (complex V). Cumulatively, this results in increased mitochondrial ATP production that fuels contraction. IMM, inner mitochondrial membrane; IMS, intermembrane space; OMM, outer mitochondrial membrane.

The mitochondrial calcium uniporter holocomplex

The mitochondrial Ca^{2+} uniporter holocomplex (uniplex) is a major pathway of mitochondrial Ca^{2+} influx that

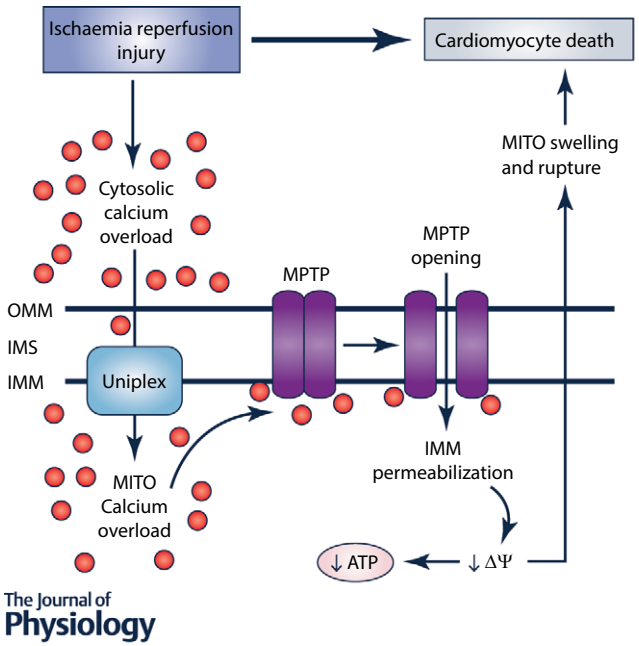

Figure 2. Ca^{2+} overload-induced cell death in the heart
Pathological conditions such as cardiac ischaemia–reperfusion injury result in cytosolic Ca^{2+} overload. The uniplex transports Ca^{2+} into the mitochondrial matrix, resulting in activation and opening of the MPTP. MPTP opening causes inner mitochondrial membrane (IMM) permeabilization, loss of mitochondrial membrane potential ($\Delta\Psi$) and impaired ATP synthesis. IMM permeabilization also causes mitochondrial (MITO) swelling and rupture, which ultimately results in cardiomyocyte death. IMS, intermembrane space; OMM, outer mitochondrial membrane.

facilitates the membrane potential-dependent transport of Ca^{2+} ions across the mitochondrial inner membrane into the matrix (Pradhan *et al.* 2010; Kamer *et al.* 2014). The uniplex is a 480 kDa multimeric channel consisting of pore-forming MCU subunits (Baughman *et al.* 2011; De Stefani *et al.* 2011) as well as the regulatory elements MICU1 (Perocchi *et al.* 2010; Mallilankaraman *et al.* 2012*b*), MICU2 (Plovanich *et al.* 2013), EMRE (Sancak *et al.* 2013), MCUb (Raffaello *et al.* 2013) and SLC25a23 (Hoffman *et al.* 2014). MCU, a dual pass transmembrane protein of the inner mitochondrial membrane, is central to the uniplex as it oligomerizes to form the Ca^{2+} transducing pore of the complex (Baughman *et al.* 2011; De Stefani *et al.* 2011; Raffaello *et al.* 2013). The Ca^{2+} selectivity filter of MCU lies within a highly conserved intermembrane space facing a loop containing a DIME motif that links the two transmembrane helices (Baughman *et al.* 2011). Key acidic residues within this motif are critical for Ca^{2+} transport as mutations at these sites render the uniplex inactive (Baughman *et al.* 2011). Computational studies have suggested that the uniplex channel is composed of MCU tetramers (Raffaello *et al.* 2013). Interestingly, however, a recent structural analysis by nuclear magnetic resonance in combination with electron microscopy of the *Caenorhabditis elegans* uniplex revealed a pentameric organization (Oxenoid *et al.* 2016). This study in *C. elegans* may have important implications for our understanding of uniplex architecture, and highlights the need for additional studies to understand the composition of this transporter in mammals and *in vivo*.

As mentioned above, in addition to MCU oligomers, models of the uniplex also include the regulatory proteins MICU1, MICU2, EMRE, SLC25a23 and MCUb. MICU1 and MICU2 are thought to serve as Ca^{2+}-sensing proteins that modulate channel opening at low and high cytosolic Ca^{2+} concentrations (Patron *et al.* 2014). EMRE has been found to regulate uniplex assembly by linking MCU to MICU1/2 (Sancak *et al.* 2013), and recent studies have also shown that EMRE can also regulate uniplex activity by serving as a matrix Ca^{2+} sensor (Vais *et al.* 2016). SLC25a23, an inner membrane Mg-ATP/P_i transporter is thought to modulate Ca^{2+} influx through interaction with MCU and MICU1 (Hoffman *et al.* 2014). MCUb was identified as an MCU paralogue that shares high sequence and structure similarity with MCU but lacks the key acidic residues that confer Ca^{2+} transport ability (Raffaello *et al.* 2013). Thus, MCUb may function by integrating into the MCU oligomeric pore to act as an endogenous repressor of uniplex-dependent Ca^{2+} transport (Raffaello *et al.* 2013). Finally, MCUR1 has also been proposed to function as a uniplex regulator (Mallilankaraman *et al.* 2012*a*), but this finding has been controversial (Paupe *et al.* 2015) and MCUR1 may serve as a link between the uniplex and the respiratory chain (Tomar *et al.* 2016).

In an added layer of complexity, the molecular composition of the uniplex – the expression levels of regulatory subunits, and stoichiometry of Ca^{2+} transducing to inhibitory subunits within the uniplex pore – may vary between tissues (Raffaello *et al.* 2013). Thus, to date, the precise molecular composition of the cardiac uniplex is unknown. Clearly, work aimed at understanding the complete molecular architecture of the uniplex is needed, as MCU's oligomerization states may have important implications for the stoichiometry and interactions with regulatory subunits, thereby impacting the design of pharmacological agents to modulate uniplex function in the heart.

The MCU and the regulation of cardiac energetics

Cardiac function is driven by Ca^{2+} cycling and excitation–contraction coupling that require energy (Bers, 2002). The fact that mitochondria are the major suppliers of ATP for contraction, combined with the fact that Ca^{2+} signalling regulates mitochondrial energy output, points to the importance of the mitochondrial Ca^{2+} microdomain in linking cardiac energy supply to contractile demand (Balaban, 2009). *In vivo* models of MCU deletion and inactivation have greatly illuminated the contribution of MCU, the uniplex and mitochondrial Ca^{2+} dynamics to the regulation of cardiac energetics.

Mouse models of uniplex inhibition, either by constitutive gene disruption using a genetrap strategy (MCU-constitutive knockout (KO); Pan *et al.* 2013), or via a targeted loxP-Cre strategy allowing for the cardiac specific induction of MCU deletion (MCU cardiac KO; Kwong *et al.* 2015; Luongo *et al.* 2015), or through transgenic overexpression of a dominant negative MCU in the heart (dnMCU; Wu *et al.* 2015), have resulted in inhibition of mitochondrial Ca^{2+} influx. Despite this across-the-board inhibition of uniplex-dependent Ca^{2+} influx observed amongst the models, interestingly, the effects of *in vivo* MCU deletion on matrix Ca^{2+} content varied. MCU-constitutive KO mitochondria displayed depressed matrix Ca^{2+} content (Pan *et al.* 2013) while matrix Ca^{2+} levels in the MCU cardiac KO mitochondria were unchanged (Kwong *et al.* 2015; Luongo *et al.* 2015), suggesting differential effects of long term *versus* acute MCU deletion on mitochondrial Ca^{2+} homeostasis.

With regards to mitochondrial bioenergetics, MCU deletion from cardiac mitochondria did not affect mitochondrial energetics at baseline, suggesting that MCU is dispensable for mitochondrial function in unstimulated conditions (Pan *et al.* 2013; Kwong *et al.* 2015). The impact of MCU deletion on mitochondrial energetics was revealed in the presence of Ca^{2+}. While control mitochondria increased oxygen consumption with Ca^{2+} stimulation, this response is abrogated with MCU deletion (Pan *et al.* 2013; Kwong *et al.* 2015). Significantly, this impairment

in Ca^{2+}-stimulated respiration translated into an inability of MCU-deleted cardiac mitochondria to upregulate ATP synthesis in response to Ca^{2+} signalling (Kwong *et al.* 2015).

When does the heart require Ca^{2+}-stimulated upregulation of mitochondrial energy production? During the fight or flight response, catecholamine stimulation initiates a cascade of events that ultimately lead to elevated cytosolic Ca^{2+} that drives enhanced contraction (Katz & Lorell, 2000). Thus, increased mitochondrial energy production is required to support this elevated contractile response. Studies with dnMCU transgenic expression revealed that MCU plays an important role in cardiac pacemaker cell function. With dnMCU-mediated MCU inhibition, stimulation of pacemaker cells with the β-adrenergic agonist isoproterenol resulted in inhibited mitochondrial Ca^{2+} influx and impaired heart rate acceleration, suggesting an impairment in the coordination of the fight or flight response (Wu *et al.* 2015). In addition to pacemaker cells, MCU was shown to have an expanded role in cardiac fight or flight regulation as cardiomyocyte-specific deletion of MCU resulted in impaired isoproterenol-induced increase in mitochondrial matrix Ca^{2+} content (Kwong *et al.* 2015), which resulted in blunted isoproterenol-stimulated respiration (Kwong *et al.* 2015; Luongo *et al.* 2015), decreased isoproterenol-induced NADH production (Luongo *et al.* 2015) and depressed cardiac contractility in response to acute isoproterenol challenge (Kwong *et al.* 2015; Luongo *et al.* 2015). It should be noted, however, that the MCU-constitutive KO animals did not demonstrate similar functional deficits in response to adrenergic stress (Holmstrom *et al.* 2015), once again highlighting the differential effects of constitutive global MCU deletion *versus* induced acute MCU deletion in the adult heart.

Importantly, with prolonged catecholamine stimulation, the differences between control and MCU cardiac KO animals were abolished (Kwong *et al.* 2015) as over an expanded time frame, MCU cardiac KO animals were able to match the matrix Ca^{2+} accumulation, mitochondrial respiration, and cardiac function of controls (Kwong *et al.* 2015). Interestingly, reminiscent of the observations in mice, isolated guinea pig cardiomyocytes treated with Ru360 and treated with isoproterenol displayed a slight trend for reduced cytosolic Ca^{2+} with acute adrenergic stimulation, but this difference was abrogated over time (Kohlhaas *et al.* 2010). Strikingly, however, guinea pig cardiomyocytes displayed a sustained depressed NADPH production with prolonged isoproterenol, suggesting a sustained bioenergetic mismatch (Kohlhaas *et al.* 2010), a difference that could potentially be attributed to the differences in model systems (discussed further below). Nevertheless, translating the deficit in the acute isoproterenol response observed in the mouse heart to the whole animal, MCU cardiac KO animals displayed impaired running capacity when challenged by an enforced immediate sprint protocol, yet performed like controls when afforded a long and slow warm-up (Kwong *et al.* 2015).

Together, the studies using targeted genetic approaches support the concept that MCU and the uniplex transduce a fast Ca^{2+} signal that allows mitochondria to match energy output to increased contractile demand and highlight the possibility of modulating mitochondrial Ca^{2+} influx to enhance cardiac function. An important point of consideration, however, as we grow our understanding of MCU's role in cardiac physiology, is that at present, much of our knowledge is based on mouse modelling. As the resting heart rate in mice (450–750 beats min^{-1}) is much higher than that of larger animals like guinea pigs (200–300 beats min^{-1}) and humans (60–100 beats min^{-1}), murine cardiac mitochondria are challenged with much more Ca^{2+} than that of larger animals. As such, the functions of mitochondrial Ca^{2+} signalling, and perhaps even uniplex activity and regulation, may be very different between excitable *versus* non-excitable tissues, and also across species. Indeed, it has been demonstrated that uniplex activity varies between tissues and that cardiac mitochondria display low current density as compared to mitochondria from other sources (Fieni *et al.* 2012). This decreased current density may be one mechanism whereby cardiac mitochondria can withstand the cyclical Ca^{2+} elevations of excitation–contraction coupling to prevent mitochondrial Ca^{2+} overload (Fieni *et al.* 2012). As an extension of this idea, one possibility might be that cardiac uniplex activity and regulation differ amongst species, thereby accounting for species-specific differences in cardiac function. At present, data from the mouse models point to the possibility that uniplex function is restricted to acute mitochondrial Ca^{2+} influx, which opens the door for alternative MCU-independent mechanisms of mitochondrial Ca^{2+} import. What are the molecular identities of these putative additional mitochondrial Ca^{2+} transporters? Indeed, mitochondrial Ca^{2+} current has been detected in cells with MCU knockdown (Bondarenko *et al.* 2013) and both the transient receptor potential canonical 3 (TRPC3) and mitochondria localized ryanodine receptor 1 (RyR1) have been suggested to play a role in mitochondrial Ca^{2+} influx (Ryu *et al.* 2010; Feng *et al.* 2013). But certainly, additional studies are needed to determine whether TRPC3 and RyR1 contribute to cardiac mitochondrial Ca^{2+} handling, whether additional yet unidentified mechanisms contribute to mitochondrial Ca^{2+} influx and cardiac energetics, and finally, how these mechanisms might interact with the uniplex.

MCU's role in calcium overload-induced death in the heart

As mentioned above, mitochondrial Ca^{2+} signalling not only impacts energetics but can also engage cellular death pathways. Under physiological conditions, the mitochondrial inner membrane is impermeable to most small molecules and ions, and the electron transport chain establishes an electrochemical gradient that is harnessed by the ATP synthase to generate ATP. Under pathological conditions, however, it is recognized that mitochondrial Ca^{2+} overload triggers the opening of the mitochondrial permeability transition pore, which allows for free passage of solutes < 1.5 kDa in size (Haworth & Hunter, 1979; Hunter & Haworth, 1979; Kwong & Molkentin, 2015). This results in inner membrane permeabilization, membrane potential collapse, ATP synthesis impairment, mitochondrial swelling, rupture and cell death (Kwong & Molkentin, 2015). This mitochondrial Ca^{2+}–MPTP signalling axis has long been postulated to contribute significantly to the cardiomyocyte death observed following ischaemia–reperfusion injury. MPTP inhibition either pharmacologically or via genetic ablation of MPTP constituents has shown great promise in preventing cardiomyocyte death (reviewed in Kwong & Molkentin, 2015). Since the uniplex is a major mode of mitochondrial Ca^{2+} influx, uniplex inhibition represents an attractive alternative means to prevent Ca^{2+} overload activation of the MPTP and subsequent death in the heart. Indeed, pharmacological inhibition of the uniplex with the MCU-specific inhibitors ruthenium red and its derivative Ru360 have been highly effective in limiting cardiac ischaemic injury (Garcia-Rivas *et al.* 2006; Zhang *et al.* 2006).

While studies using ruthenium red and Ru360 to inhibit mitochondrial Ca^{2+} have been overwhelming in their support for uniplex inhibition as a means to prevent Ca^{2+} overload-induced cell death both *in vitro* and *in vivo* (Groskreutz *et al.* 1992; Dessi *et al.* 1995; Garcia-Rivas *et al.* 2006; Zhang *et al.* 2006; Qiu *et al.* 2013), studies using gene targeted mouse models of uniplex inactivation have been less clear. MCU-constitutive KO mice display resistance to Ca^{2+} overload-induced MPTP activation, but surprisingly, no protection against *in vivo* cardiac ischaemia–reperfusion injury (Pan *et al.* 2013). In contrast, the MCU cardiac KO mice with adult induction of MCU ablation not only displayed inhibited Ca^{2+}-stimulated MPTP opening, but also showed greatly reduced cardiomyocyte death following *in vivo* cardiac ischaemia–reperfusion injury (Kwong *et al.* 2015; Luongo *et al.* 2015).

What accounts for the differences in death between these two animal models? The answer may lie in the consequences of constitutive *versus* acute MCU deletion – which to date, are not fully understood. The MCU-constitutive KO mice display changes in metabolism that cause a shift away from oxidative pathways, as well as insensitivity to the MPTP inhibitor cyclosporine A (Pan *et al.* 2013). Similar alterations were not observed in an MCU cardiac KO model (Kwong *et al.* 2015; Luongo *et al.* 2015). These findings hint at the possibility that long-term MCU deletion may cause global gene expression changes that we have not fully catalogued, via mechanisms that to date are unknown. Since acute MCU inhibition is highly protective against Ca^{2+} overload-induced death, controlled and reversible inhibition of mitochondrial Ca^{2+} influx may be a strategy to prevent cardiomyocyte demise following ischaemia–reperfusion injury, and studies directly comparing the two models will be important in the design of uniplex-targeted therapeutics.

A role for MCU beyond mitochondria?

As discussed above, the uniplex has well recognized roles in regulating cardiac biology through its immediate actions on mitochondrial energetics and mitochondrial death pathways. In addition to proximal effects on mitochondria, however, does uniplex signalling extend beyond the confines of mitochondrial biology to regulate broader aspects of cellular function? Hints at a wider role come from the MCU-constitutive KO mice, as these were 30% smaller, and displayed depressed pyruvate dehydrogenase activity and chronic acidosis (Pan *et al.* 2013), suggesting the possibility that chronic inhibition of MCU signalling results in widespread metabolic changes that intersect with global growth pathways. Recent work on MCU in skeletal muscle also supports a connection between the uniporter and growth pathways as adeno-associated virus (AAV)-mediated MCU overexpression enhanced mitochondrial Ca^{2+} influx and caused myofibre hypertrophy, while AAV delivery of MCU shRNA inhibited matrix Ca^{2+} influx and resulted in myofibre atrophy (Mammucari *et al.* 2015). Further, AAV–MCU activated both the Akt–GSK3 α/β–4E-BP1 growth signalling axis and the PCG1α mitochondriogenesis pathway, while AAV–shMCU caused the opposite effect (Mammucari *et al.* 2015). Collectively, these findings suggest that the uniplex may link mitochondrial energetics to global growth programmes. These findings, however, need to be validated in the MCU global knockout and loxP-targeted mouse models, and it remains to be determined if the uniplex can control similar pathways in the heart.

Perspectives

The study of mitochondrial Ca^{2+} dynamics has undergone a molecular revolution. Once known only as a phenomenon that could be inhibited by ruthenium red and its derivatives, the mitochondrial Ca^{2+} import

machinery has now grown into the multiprotein assembly we now know as the uniplex. Yet, the molecular landscape of the uniplex may still be evolving. With a growing list of uniplex regulatory subunits and regulators, as well as a potential new framework for the uniplex pore structure observed in invertebrates, understanding the precise molecular architecture of the uniplex in the mammalian heart, and defining regulators that impact cardiac mitochondrial function will be of great importance as we move to developing new tools to modulate uniplex function. The mouse models of MCU inactivation have revealed roles for the uniplex in regulating two very different pathways: cardiac metabolic contraction coupling, and Ca^{2+} overload-induced death. Moving forward, it will be important to understand if the uniplex's role in physiological Ca^{2+} signalling can be separated from its pathological roles and if there are regulatory elements that are specific for energy production *versus* death. Studies on the constitutive and inducible cardiac MCU deletion models have also illuminated differences between long-term and short-term inhibition of uniplex-dependent mitochondrial Ca^{2+} influx. Certainly studies thus far support acute uniplex inhibition as a therapeutic avenue of great interest to restrict cardiomyocyte loss following ischaemia–reperfusion injury. Therefore, understanding the ramifications of long term uniplex inhibition as well as how mitochondrial Ca^{2+} signalling influences global cardiac gene expression will be critical as we move towards the goal of developing new strategies to modulate uniplex function to enhance cardiac function by augmenting mitochondrial energetic output while limiting cardiomyocyte death.

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Additional information

Competing interests

None declared.

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