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Review article

The mitochondrial calcium uniporter: Mice can live and die without it

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ABSTRACT

Calcium is of critical importance to mitochondrial and cell function, and calcium signaling is highly localized in the cell. When stimulated, mitochondria are capable of rapidly taking up calcium, affecting both matrix energetics within mitochondria and shaping the amplitude and frequency of cytosolic calcium "waves". During pathological conditions a large increase in mitochondrial calcium levels is thought to activate the mitochondrial permeability transition pore, resulting in cell death. The protein responsible for mitochondrial calcium uptake, the mitochondrial calcium uniporter (MCU), was identified in 2011 and its molecular elucidation has stimulated and invigorated research in this area. MCU knockout mice have been created, a variety of other regulators have been identified, and a disease phenotype in humans has been attributed to the loss of a uniporter regulator. In the three years since its molecular elucidation, further research into the MCU has revealed a complex uniporter, and raised many questions about its physiologic and pathologic cell roles. This article is part of a Special Issue entitled 'Review Article Mitochondria'.

Contents

1. Introduction

The clinical significance of calcium has been appreciated for centuries, since Ringer first discovered in 1883 that the addition of the divalent ion could trigger contractions in cardiac myocytes [1]. Mitochondrial calcium uptake was first measured over 50 years ago, when studies in the 1960s showed that mitochondria were capable of rapidly taking up calcium [2,3]. When this occurred mitochondrial matrix concentrations of total calcium could rise by factors of 10 or more [2,4,5]. The ability of isolated mitochondria to accumulate calcium led to suggestions in the

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late 1970s that mitochondria might contribute to the regulation of cytosolic calcium. David Nicholls showed that because of the relative kinetics of mitochondrial uptake by the uniporter and release by the Na–H (or Na–Ca) exchanger that mitochondria could regulate extra-mitochondrial calcium to a "set point": if extra-mitochondrial calcium was raised above the set point the mitochondria would accumulate calcium, but if extra-mitochondrial calcium was reduced below the set point, mitochondria would release calcium via the efflux pathway [6]. This concept that mitochondria regulate cytosolic calcium was challenged by studies in giant squid axon in which the cytosol could be loaded with calcium sensitive dyes such as arsenazo. These studies suggested that, under physiological conditions, cytosolic calcium did not rise to levels sufficient to support mitochondrial calcium uptake; others suggested that the role of mitochondrial calcium uptake was not to regulate cytosolic calcium, but rather to regulate mitochondrial matrix calcium and the activity of calcium sensitive mitochondrial dehydrogenases [7,8].

In the 1980s, the role of intracellular organelles in regulating cell calcium homeostasis turned away from the mitochondria and towards other organelles [9–11]. One reason for this was that baseline levels of mitochondrial calcium were found to be relatively low, and generally comparable to that of the cytosol (~100 nM), suggesting that mitochondria do not serve as reservoirs for large amounts of calcium, at least at baseline cell conditions [8,12–16]. Even with agonist stimulation or at peak contractility bulk cytosolic calcium only rose to ~1 μM, and only very transiently [12,17].

Although it was well established that the sarcoplasmic reticulum was the intracellular organelle involved in calcium release and reuptake during calcium transients, the intracellular source of agonist-induced calcium release was unknown, and mitochondria were considered a possible source. In the 1980s, several groups found that agonists that led to the generation of inositol 1,4,5 triphosphate (IP3), caused calcium release from the endoplasmic reticulum [10,11]. Other research showed that the endoplasmic reticulum had a much higher affinity for calcium than the mitochondria [18]. It was generally agreed that mitochondria did not have a major role in regulating cytosolic calcium homeostasis and that mitochondria only accumulated calcium under pathological cell death conditions associated with a massive increase in cytosolic calcium [19]. Accordingly, research turned to focus largely on the endoplasmic reticulum's role in cellular calcium handling.

1.1. Attention shifts back towards mitochondria

Attention returned to the mitochondria as a major player in cellular calcium in the 1990s, when the development of highly specific probes made it possible to demonstrate microdomains of high calcium near the mitochondria [20–22]. When channels on sarcoplasmic/endoplasmic reticulum (SR/ER) or plasma membrane opened, there was a sudden, local increase of calcium five to ten times the general cytosolic calcium concentration. Mitochondria near these microdomains of high calcium concentration were able to rapidly take up calcium. Therefore high levels of cytosolic calcium, sufficient to activate MCU did exist, in small focused areas often in close proximity to mitochondria, which were then able to accumulate calcium [23].

The concept emerged that calcium release from SR/ER exposed mitochondria to a much higher calcium concentration that what is typically present in the cytosol [24,25]. This picture also helped to reconcile the fact that mitochondrial calcium was essential for aerobic metabolism with its roles in propagating cell death: while an accumulation of calcium could cause cell death, a rapid and transient rise in calcium, the kind initiated by the brief appearance of these microdomains of high cytosolic calcium, could exist physiologically [24].

Mitochondria also appear to be docked to the ER/SR at designated signaling sites, ensuring their proximity and their ability to utilize these small, locally potent releases of calcium [26]. It was shown that the if the tethers between the ER/SR and mitochondria were tightened, mitochondria became more prone to calcium overload, mPTP opening and subsequent cell death, presumably because of their increased exposure to microdomains of high cytosolic calcium (see Section 2b) [23,27].

2. (Patho)physiological roles of mitochondrial calcium

Balanced calcium uptake by the mitochondria is essential: at appropriate levels, it can stimulate important metabolic processes such as activation of mitochondrial dehydrogenases, but higher mitochondrial calcium can be detrimental for a cell, initiating cell death pathways such as apoptosis and necrosis. Mechanisms for altering mitochondrial calcium levels, and maintaining homeostasis, are therefore essential for both aerobic metabolism and cell survival [4].

2.1. Metabolism

Mitochondria are classically referred to as the powerhouse of the cell: provided with oxygen and reducing equivalents, respiring mitochondria are able to produce ATP and maintain a membrane potential. Three mitochondrial matrix dehydrogenases essential for ATP production are activated by calcium: pyruvate dehydrogenase, alpha-ketoglutarate, and isocitrate-dehydrogenase [28]. The stimulation of these dehydrogenases by calcium increases NADH availability, and therefore the flow of electrons down the respiratory chain: mitochondrial calcium increases mitochondrial ATP production [29]. Calcium is also known to activate several complexes of electron transport [30,31].

2.2. Cell death

Mitochondria are capable of rapidly taking up calcium, but at very high levels, their ability to buffer that calcium can be overwhelmed. When this occurs, pathological calcium concentrations are reached, and a large conductance channel known as the mitochondrial permeability transition pore (mPTP) opens in the inner mitochondrial membrane [32–34]. First formally described by Haworth and Hunter in 1976, this pore has since been implicated in a multitude of cell death pathways, including cardiac and neuronal cell death, hepatotoxicity, and nervous and muscular dystrophies [35]. This pore has since been implicated in a multitude of cell death pathways, including cardiac and neuronal cell death, hepatotoxicity, and nervous and muscular dystrophies. The process appears to begin with an oxidative stress and/or ATP depletion, which is followed by mitochondrial calcium loading to pathologically high levels, inducing the mPTP to open [36]. When the mPTP opens there follows a collapse of the mitochondrial membrane potential and a subsequent bioenergetic crisis. MPT-dependent mitochondrial swelling occurs, and cell death rapidly ensues [37]. Opening of the mPTP appears to play a fundamental role in reperfusion injury in the heart [32–34,38]. The low pH during ischemia is known to inhibit the mPTP, but as cytosolic pH is restored on reperfusion the mPTP opens [38]. In both I/R injury and other forms of mPTP induced cell death such as neuronal glutamate toxicity, blocking of either the mPTP or the reduction of mitochondrial calcium uptake appears to be protective, suggesting that mitochondrial calcium uptake may be a potential site for therapeutic intervention [39,40].

3. MCU identified

Although it had been clear for decades that mitochondrial calcium levels were involved in the regulation of processes ranging from aerobic metabolism to cell death, the actual protein responsible for calcium uptake into the mitochondria had not been identified. Because the outer membrane of the mitochondria has channels such as the voltage dependent anion channel (VDAC) that render it freely permeable to calcium, the MCU was proposed to be on the inner membrane of the mitochondria, but its molecular identity was unknown. Evidence suggested that the MCU would be i.) highly selective, ii.) sensitive to ruthenium red, (RuR) and iii.) have low affinity for the cation [41,42]. The driving

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