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Review

Molecular diversity and pleiotropic role of the mitochondrial calcium uniporter

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ABSTRACT

The long awaited molecular identification of the mitochondrial calcium uniporter (MCU) in 2011 has opened an exciting phase in the study of mitochondrial calcium homeostasis. On the one hand, MCU proved to be the core of a complex signaling system, composed of a channel moiety (MCU itself and the related MCUb protein) and a family of essential regulators (the MICUs, MCUR, EMRE). On the other hand, the availability of molecular information and tools opened the possibility of directly altering mitochondrial calcium homeostasis in cell cultures or intact organisms, thus obtaining new insight into its role in physiological and pathological events. We will review here these exciting advancements, summarizing the current knowledge of the molecular composition of the MCU complex and of its role in shaping mitochondrial and cytosolic [Ca^{2+}] signals.

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1. Introduction

The occurrence of rapid calcium fluxes across the inner mitochondrial membrane of energized mitochondria was observed in the early 1960s and then rationalized when the chemiosmotic theory of Peter Mitchell provided the thermodynamic basis for the phenomenon: the large electrical gradient ($\Delta\Psi$) established by the activity of the respiratory chain. At the same time, the notion that calcium influx depends on membrane potential implies that calcium at steady state should reach electrochemical equilibrium. Given a resting cytosolic [Ca^{2+}]around 100 nM and a membrane potential of −180 mV, the predicted [Ca^{2+}] of the mitochondrial matrix ([Ca^{2+}]$_{mt}$) should reach at least 100 mM, in contrast with all values obtained experimentally.

It soon became clear that mitochondrial calcium homeostasis is based on the equilibrium between the activity of an electrogenic pathway, termed the "mitochondrial calcium uniporter" (MCU) and of extrusion pathways, demonstrated to act as exchangers (with either Na+ or H+), which altogether prevent the system from reaching the electrochemical equilibrium. Although the structure of the MCU remained elusive for a very long time, some functional features were known early on, such as its inhibition by Ruthenium Red and lanthanum ions, as well as its V_{max} and affinity for Ca^{2+} [1,2]. As to its affinity in situ, based on the study of Ca^{2+} uptake by isolated mitochondria in physiological conditions of [Mg^{2+}], pH and ionic strength, it was reported to be in a concentration range higher than 25 μM. Thus, especially when the accurate calibration of fluorescent indicators allowed to estimate that cytosolic [Ca^{2+}] varies between ≈0.1 μM (in resting conditions) and ≈1–5 μM in the peak response to cellular stimulation, the role of mitochondrial Ca^{2+} uptake in cell physiology was questioned. Overall, the view that mitochondria accumulate calcium only under pathological conditions of cellular Ca^{2+} overload became prevalent based on all evidence available at the time, mostly in isolated mitochondria.

This consensus was reversed when probes became available for directly measuring [Ca^{2+}]$_{mt}$ in intact, living cells. In particular, when the calcium-sensitive photoprotein aequorin was specifically targeted to the mitochondrial matrix, it became clear that large [Ca^{2+}]$_{mt}$ increases occur dynamically in parallel with agonist-induced [Ca^{2+}]$_{cyt}$ increases [3]. The apparent discrepancy with the low affinity of the MCU was resolved by the demonstration that cell architecture brings mitochondria in close contacts with the source of the [Ca^{2+}]$_{cyt}$ rise, i.e. in proximity of the Ca^{2+} channels of the endoplasmic reticulum (ER) or of the plasma membrane [4]. Mitochondrial Ca^{2+} uptake appears thus in most cases triggered by microdomains where MCU is exposed to [Ca^{2+}] over one order of magnitude higher than those in the bulk cytosol. These conclusions led to a novel dynamic view of the structural relationship between the ER calcium stores and mitochondria. The concentrations measured in intact cells with recombinant aequorin not only matched

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the kinetic properties of the uniporter, but also those of key mitochondrial enzymes (see below), thus drawing a regulatory axis from cell activation by extracellular agonists to second messengers and cell metabolism.

Although MCU-mediated Ca^{2+} uptake was precisely characterized in a large number of cell types and conditions, its molecular identity remained a black box for decades, even after mitochondrial Ca^{2+} homeostasis reached a central stage in biology. Indeed, the fact that the mitochondrial calcium uniporter could be a gated channel rather than a carrier had been postulated 30 years earlier by the group of GF Azzone and demonstrated in 2004 by D. Clapham and coworkers using isolated mitoplasts [5]. The study was of great importance as it highlighted fundamental properties of the MCU channel, such as the very high selectivity for calcium ions, which essentially prevents uncoupling of mitochondria due to permeation of other cations. Furthermore, the calcium concentrations needed to activate the channel are indeed much higher than those measured under resting conditions in the cytosol, leading to a negligible open probability of the channel and minimal calcium leak, thus reducing energy drainage in non-stimulated cells.

The molecular era of the mitochondrial calcium uniporter began in 2011, as our group and that of V. Mootha simultaneously reported the identification of the predicted transmembrane protein CCD109A as the pore-forming subunit of the mitochondrial calcium uniporter [6,7]. More recently, a number of novel components have been added, using comparative genomics-based approaches, to this elementary channel moiety. We will henceforth refer to the structural assembly of proteins underlying the mitochondrial calcium uniporter complex as the "MCU complex". The structure of the complex will be described in Section 3. A main focus of this review is to relay the complex and dynamic assembly modules of the MCU complex to the pleiotropic role of mitochondrial calcium in cell physiology and pathology

2. Mitochondrial calcium in pathophysiology

The influx of Ca^{2+} into the mitochondrial matrix is a control hub for many different aspects of cell physiology. Although the basic machinery responsible for mitochondrial calcium homeostasis is very similar in most metazoans, the dynamics of Ca^{2+} influx are very diverse depending on the cell type and biological context. $[Ca^{2+}]_{mt}$ fluctuations are linked to many functional outputs, which range from the regulation of bioenergetics to cell death. The amplitude and duration of $[Ca^{2+}]_{mt}$ transients can be, accordingly, very different. Furthermore, the number of mitochondria and, thus, the fraction of cell volume they represent, are highly variable in different cell types, mostly in connection with their metabolic profile. Although relative mitochondrial volume per se is not a direct function of metabolic efficiency, excitable cells like neurons, cardiomyocytes and skeletal muscle fibers, which rely on oxidative phosphorylation for ATP production, show a higher mitochondrial content.

The spatial distribution of mitochondria is also a parameter that diversifies cell types in terms of Ca^{2+} uptake, since ER/mitochondria contact sites play an essential role in the activation of the MCU, as discussed above. In support of its high energy demand, skeletal muscle displays a tight coupling between sarcoplasmic reticulum (the calcium store of muscle cells) and mitochondria, with numerous sites of juxtaposition dictated by the rigid geometry of myofibrils. In neurons, mitochondria located at the synapse or in axons and dendrites are secluded from the endoplasmic reticulum but in close contact to the plasma membrane. In this case, functional coupling between voltage-dependent calcium channels and mitochondria ensures the efficiency of the influx process.

We will describe three major different functions of mitochondrial calcium highlighting the pleiotropic mode of action of the MCU complex: (i) metabolic regulation, (ii) high capacity calcium buffering and (iii) cell damage and death.

2.1. Metabolic regulation

The citrate cycle and respiratory chain yield 36–38 molecules of ATP from processing a glucose molecule, compared to only two obtained by anaerobic glycolysis. Calcium ions in the mitochondrial matrix control and coordinate various steps of these processes, directly linking cell activation to energy needs. Indeed, many rate-limiting enzymes of oxidative metabolism, including three key dehydrogenases, are calcium dependent [8]. Isocitrate- and α-ketoglutarate dehydrogenase directly bind Ca^{2+} through EF-hand motifs, whereas pyruvate dehydrogenase is activated via a Ca^{2+}-dependent dephosphorylation step. This way, the higher ATP demand of stimulated cells (e.g. a contracting muscle or a secreting endocrine or exocrine cell), through the activatory signal conveyed by the Ca^{2+} rise is met by an increased supply of reducing equivalents, NADH or FADH, to the respiratory chain. A direct link between $[Ca^{2+}]_{mt}$ increases and ATP production has been demonstrated using two different photoproteins recombinantly targeted to the mitochondrial matrix, aequorin to measure $[Ca^{2+}]$ (see Section 1) and firefly luciferase as a reporter of ATP levels [9]. In HeLa cells, stimulation with histamine caused large increases in both mitochondrial calcium and ATP. This effect was blunted when mitochondrial calcium was buffered, revealing a direct cause-effect relationship between the two concomitant events. The same effect was also observed in specialized cell types such as skeletal myotubes and pancreatic beta cells, which, unlike HeLa cells, rely almost entirely on oxidative metabolism [9,10]. It should also be emphasized that, in pancreatic beta cells, ATP production also plays a fundamental role in cell signaling, since the ATP/ADP ratio controls the K_{ATP} channel and thus insulin secretion. Indeed, if mitochondrial calcium uptake is inhibited by silencing the MCU subunit of the uniporter, glucose-mediated insulin secretion is abolished [11].

The fundamental importance of the mitochondrial calcium-energy metabolism axis is also revealed by its role in pathological events. In the context of neurodegenerative diseases new pharmacological strategies are emerging which target mitochondrial respiratory function to design therapeutic interventions aiming at neuroprotection. Despite the large diversity in the molecular defects underlying these pathologies, perturbation of mitochondrial calcium homeostasis and ATP synthesis, as well as ROS production, are shared by most of them, so that a common pharmacological strategy could be devised. A particularly relevant group of neurodegenerative diseases that would be targeted by this approach is that of mitochondrial disorders linked to mutations in different subunits of mitochondrial complex I (NADH:ubiquinone oxidoreductase). Indeed, experiments performed in cybrids harboring mitochondrial tRNA mutations or skin fibroblasts derived from patients with mutations in different subunits of complex I show a reduction in bradykinin-stimulated mitochondrial calcium uptake and a concomitant decrease in ATP production, correlating with the residual activity of complex I [12,13].

Targeting the basic cellular machinery regulating mitochondrial calcium influx and energy production could thus be a way to tackle the multi-systemic nature of this heterogeneous class of diseases.

2.2. High capacity calcium buffering

In many cell types, mitochondria amount to a fairly large cell fraction and thus represent a potential void volume where excess cytosolic calcium can be redirected in case of need. Given the high