CALCIUM HOMEOSTASIS: THE MITOCHONDRIAL TARGET AND INTERACTION WITH HORMONES

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Abstract—Intracellular calcium regulates a wide variety of physiological processes. Mitochondria cannot be considered as a static structure, as changes in cytoplasmic Ca^{2+} concentration, resulting from activation of intracellular Ca^{2+} channels within the mitochondria, regulate several aspects of cell regulatory and signaling events. Well beyond its critical bioenergetic role in supplying ATP, mitochondria act as the centre of many diverse cellular functions, which integrate the signals between the organellae and the nucleus. In addition, a close relationship exists between mitochondrial bioenergetic pathways and calcium transport, which both may demand to activate cell signaling events. Thus, mitochondrial target in calcium homeostasis appears to be a prerequisite for proper functioning of the cell.

Keywords: Mitochondria, Calcium, Ion homeostasis, Calcium uniporter, Hormones.

I. INTRODUCTION:

Presence of cations within the various compartments of mitochondria is critical in the physiology of mitochondria. Among this, mitochondrial calcium (Ca^{2+}) regulates several cellular processes, and its concentration is, in turn regulated by various channels, pumps and exchangers. In particular, the endoplasmic reticulum (ER) was considered as the dynamic Ca^{2+} regulator in the cell. However, mitochondria were considered to play a major role in intracellular Ca^{2+} homeostasis at the beginning of the 1960s when it was discovered that they could rapidly take up large calcium loads. Now it has been well documented that mitochondria have been envisaged as sinks for intracellular calcium and thus have a vital role in regulating cytosolic calcium concentration and thus controls the energetic metabolism of the cells [3].

There are zones of close contact between the endoplasmic reticulum (ER) and mitochondria called MAMs (Mitochondria Associated Membranes) crucial for a correct communication between the two organelles, including the selective transmission of Ca^{2+} signals from the ER to mitochondria. Therefore, a spatially and functionally coupled transport of Ca^{2+} signals occurred in the ER and mitochondria [21]. Series of work demonstrated that Ca^{2+} release from the ER results in cytosolic Ca^{2+} increases that are paralleled by similar or even larger cycles of mitochondrial calcium uptake, and subsequent release [10]. Similarly, it has been evident that both the calcium and mitochondria showed a deeply intertwined regulation, Ca^{2+} regulates mitochondrial functions, while mitochondria shape Ca^{2+} dynamics, deregulation of either Ca^{2+} or mitochondrial signaling leads to altered physiological response of the cell or even results in cell death [10]. Keeping in this view, the present review article examines the perspective of mitochondrial calcium transport in the historical framework of the early studies.

II. THE MOVEMENT OF CALCIUM IN AND OUT OF MITOCHONDRIA

Mitochondria contain two membranes, an inner membrane folded into cristae that harbor the respiratory chain complexes, and an outer membrane permeable to solutes. The control of ion gradients across the inner membrane of mitochondria is central to bioenergetics and studies suggested that mitochondria have evolved a sophisticated array of carriers, ion channels, and transporters to move ions and metabolites across their inner membrane [7]. Similar to other cations, calcium entry into this organelle requires that the ion traverses both the outer and inner mitochondrial membrane (IMM). Subsequent studies have demonstrated that passage of calcium through the ion-impermeable IMM requires the large membrane potential difference [14] and this negative transmembrane potential is generated by the pumping of electrons through the electron transport chain present in the inner membrane, provides a huge driving force for the entry of calcium into the mitochondrial matrix. Subsequent physiological and biophysical studies identified that large amounts of calcium could rapidly enter the mitochondrial matrix through this transport mechanism [15], [31]. These observations, along with observations that entry of calcium was not directly coupled to the movement of another ion [37] established that mitochondrial calcium uptake occurred through a specific channel termed the mitochondrial calcium uniporter (mCU), that could bind calcium with nanomolar affinity [20], [2], catalyzes the rapid and passive entry of Ca^{2+} into mitochondria. This property of mitochondria allows Ca^{2+} transporters within the inner membrane to move Ca^{2+} ions across mitochondria, thus altering the cytosolic concentration of an ion that is central to cell signaling [7].

Ca^{2+}transport across the mitochondrial inner membrane is facilitated by transporters as well as through the Ca^{2+}-induced mitochondrial permeability transition pore (PTP). Previous reports suggested that there are two modes of inward transport, referred to as the Ca^{2+}uniporter and the rapid mode or RaM and two distinct mechanisms mediating outward transport, which are not associated with the PTP, referred to as the Na^{+}-dependent and the Na^{+}-independent Ca^{2+}efflux mechanisms [15]. Chalmers and Nicholls (2003) proposed that the fluxes of Ca^{2+} influence the fluxes of other ions. In this chain of events, calcium enters via the uniporter (mCU) and leaves mitochondria in exchange for sodium ions, a process catalyzed by the Na^{+}/Ca^{2+}exchanger (mNCX) [5]. Sodium ions are then extruded Ca^{2+}enters mitochondria via the uniporter (mCU), and exits mitochondria in exchange for Na^{+} via the Na^{+}/Ca^{2+}exchanger (mNCX), serve to maintain the proper electrical and chemical gradients in mitochondria by keeping ions and other factors in the right balance between the inside and outside of mitochondria. Similarly, Na^{+} leaves mitochondria in exchange for H^{+} *via* the Na^{+}/H^{+} exchanger (mNHE), and H^{+} ions are ultimately extruded by the electron transport chain (ETC) [5].

The uniporter and the Na^{+}/Ca^{2+}exchanger process are electrogenic, both depends and alters the membrane potential of mitochondria. In contrast, the NHE is electroneutral and its activity does not depend on the mitochondrial membrane potential. The

electrogenic nature of the mCU was recognized long ago and was predicted from the unidirectional fluxes across an ion channel [2]. Importantly, the uniporter operates at high, micromolar cytosolic Ca^{2+} concentrations that are only reached transiently in cells, near Ca^{2+} release channels [35]. However, the mismatch between rapid Ca^{2+} uptake by the uniporter and slow Ca^{2+} release by the exchanger, combined with the presence of a high concentration of phosphate inside the mitochondrial matrix, endows mitochondria with the capacity to act as a temporary storage compartment for Ca^{2+}. The shuffling of Ca^{2+} by mitochondria alters the amplitude of Ca^{2+} signals as well as their spatial and temporal dimensions. By diverting Ca^{2+} ions away from the cytosol, mitochondria blunt the peak of the cytosolic Ca^{2+} elevations, while the delayed release of the Ca^{2+} that has been buffered by mitochondria creates a shoulder that prolongs the Ca^{2+} signal. Mitochondria can thus turn brief, high-amplitude Ca^{2+} transients into biphasic, low amplitude Ca^{2+} elevations, a mechanism that plays a significant role in neurons by shaping the plateau phase of the Ca^{2+} response in presynaptic terminals [40], [26].

III. PHYSIOLOGICAL ROLE OF MITOCHONDRIAL CALCIUM TRANSPORT

Mitochondrial calcium transport regulates a wide range of processes from bioenergetics to cell death. The ability of mitochondria to move Ca^{2+} ions has important implications, firstly because Ca^{2+} controls numerous functions of mitochondria, and secondly because Ca^{2+} handling by mitochondria impacts on cytosolic Ca^{2+} signals and on the activity of Ca^{2+}- dependent proteins [7]. Mitochondria both encode and decode Ca^{2+} signals and these two interrelated functions directly impact on the cell metabolism and signaling [7]. Signaling pathways allow these organelles to respond to energy demands as well as to cell growth, cell death and a variety of physiological stimuli and stresses [12]. For example, mitochondria contribute to many signal transduction pathway and are actively involved in the maintenance of Ca^{2+} entry, Ca^{2+} refilling of the endoplasmic reticulum and Ca^{2+} dependent protein folding [13].

To maintain intracellular homeostasis, mitochondria accumulate calcium. The ubiquitous and probably most important consequence of mitochondrial Ca^{2+} accumulation is to control the energetic metabolism of the cells, by directly modulating the activity of enzymes located in the matrix [3], and contributes to shaping cytosolic Ca^{2+} fluctuations, thus in turn modulating cellular functions regulated by cytosolic Ca^{2+} variations. Together, these observations supported the notion that mitochondrial calcium represented a central component of metabolic regulation. Indeed, it had been known that cells and tissues appear capable of exquisitely matching the rate of ATP production with ATP utilization such that even with large fluctuations in power output, levels of metabolic intermediates such as ATP, ADP and Pi appear unchanged [1], [16]. This has been extensively studied in tissues such as the heart or skeletal muscle that see large and acute changes in their energy utilization when, for instance, the organism goes from a resting state to a full speed sprint. Under these conditions, it has been widely believed that the entry of mitochondrial calcium augments mitochondrial ATP production to acutely match the rapid increase in ATP demand [44] and thus the transport and release of Ca^{2+} by mitochondria are coupled with energy-transducing reactions in the cell.

The control of the ion diffusion throughout the cell has been revealed another important aspect of mitochondrial Ca^{2+} signaling. However, it is becoming evident that this is only one of the effects on the cellular processes controlled by mitochondrial Ca^{2+}. This aspect seems to be a general mechanism to control the shaping of cytosolic Ca^{2+} signal [3]. While the entry of small amounts of calcium may have beneficial effects for cellular ionic and metabolic homeostasis, there is a significant amount of data demonstrating that the uptake of large amounts of Ca^{2+} can induce cell death [32], [33]. The basis for this phenomenon involves opening of the permeability transition pore (PTP). While the precise molecular makeup of the PTP has remained elusive, evidence suggests that the entry of calcium through a MCU dependent mechanism is the central mediator of PTP opening [27], [22]. Once opened, the PTP results in depolarization of the IMM leading to collapse of the mitochondrial membrane potential and thus inhibition of electron transport and mitochondrial-dependent ATP production.

Apart from cellular calcium homeostasis, increasing evidences support an active role for mitochondrial Ca^{2+} homeostasis on cell migration in different animal models. In 2013, the first evidence for a role of the pore forming MCU in cell migration was provided by investigating its function in zebrafish early development [29]. Similarly, it has been well documented that mitochondrial Ca^{2+} overload sensitizes cells to apoptotic stimuli, and this has been always viewed as a promising strategy to eliminate aberrant cells, including cancer cells, which would have otherwise escaped apoptotic death [33]. In an elegant series of experiments performed in Xenopus oocytes, Jouaville *et al*. demonstrated that mitochondrial Ca^{2+} uptake can modulate the shape and velocity of $InsP_3$ induced Ca^{2+} waves suggests that mitochondrial Ca^{2+} uptake not only serves the function of controlling organelle function, but plays an unexpected role in the control of important, often apparently unrelated events which occur in the cytosol of a living cell [18]. The role of mitochondria in modulating the activity of Ca^{2+}channels is not limited, however, to the ER. Evidence has been provided for a role of mitochondria in modulating the activity of plasma membrane channels, such as store-operated Ca^{2+} channels [17].

IV. MITOCHONDRIAL CALCIUM TRANSPORT: ROLE OF HORMONES

Mitochondrial Ca^{2+} handling plays a major role in controlling hormone synthesis and/or release by endocrine cells. Nevertheless, a broad-based review on interactive role of hormones in mitochondrial calcium transport is not presently available.

A. Mitochondrial Calcium acts as a mediator for insulin action

The role of mitochondrial Ca^{2+} accumulation in the secretion of insulin by pancreatic β cells appear to be more complex. However, dose-responsive and differential actions of insulin on mitochondrial calcium transport activities were reported by Peter *et al*. (2014). The study provides evidence for an integrative role for insulin on mitochondrial calcium transport [28]. Similarly, it has been suggested that mitochondrial calcium is also having an impact on the synthesis of factors other than ATP that couple mitochondrial metabolism to insulin secretion [23]. The regulation of cellular Ca^{2+} signals by mitochondria has been found to depend on the mitochondrial energetic status [12]. Further, insulin has been shown to reduce the influx of Ca^{2+} through receptor-operated channels and to decrease the voltage-mediated Ca^{2+} influx [28]. Likewise, in pancreatic β-cells, ATP acts as a signaling molecule initiating plasma membrane electrical activity linked to Ca^{2+} influx, which triggers insulin exocytosis [30]. The study demonstrated that silencing of mCU in insulin-releasing cells decreases mitochondrial Ca^{2+} uptake.

B. *Thyroid hormones can regulate mitochondrial calcium transport:*

Thyroid hormones exert profound effects on the energy metabolism and mitochondria, by virtue of their biochemical functions, are a natural candidate as a direct target for the calorigenic effects of thyroid hormones [11]. It is interesting to consider that both thyroid hormones and Ca^{++} transport activity are interacting with the energetic metabolism by means of phosphorylation and substrate oxidation mechanism [6]. The authors studied the effects of thyroid hormones and their structural analogues on the mitochondrial calcium transport activities and reported that the thyroid hormones, 3, 5, 3' L-triiodothyronine (LT3) and 3, 5, 3'5' L-tetraiodothyronine (LT4) at physiological intracellular concentrations between 7.2 and 9 nM, decouple total Ca^{++} transport, as well as inhibit the passive transport of Ca^{++}, either due to oxidation of pyruvate, malate or succinate or after inhibition with rotenone. The effect of the thyroid hormones and of their structural analogues also revealed that the mitochondrial calcium transport may be influenced both by a stereospecific interaction between hormones and protein ligands and by a lipophilic chaotropic action on the mitochondrial membranes lipids [6] and thus it can regulate the biosynthesis of the mitochondrial calcium uniporter [34].

C. *Dynamic regulation of mitochondrial calcium transport by cortisol and aldosterone*

Cortisol and aldosterone biosynthesis occurs via a series of metabolic reactions that occur in ER and mitochondria. However, little information is available on the mechanism of actions of steroid hormones on mitochondrial calcium transport. Kimberg and Goldstein (1966) demonstrated the binding of calcium by liver mitochondria of rats treated with steroid hormones [19]. Their experiment suggested that cortisol treatment interferes with the utilization of ATP or respiratory substrate necessary to support mitochondrial calcium binding *in vitro*. Mitochondria isolated from animals treated with cortisol bind less calcium than do mitochondria from control animals under conditions in which binding is supported either by adenosine triphosphate alone or primarily by a respiratory substrate. Treatment with other glucocorticoids (prednisone and cortisone) likewise diminishes substrate-dependent calcium binding to mitochondria *in vitro*. The effect of cortisol treatment on diminishing ATP-dependent calcium binding is potentiated by the addition of oligomycin *in vitro* [19]. In aldosterone producing cells, mitochondrial Ca^{2+} accumulation is a key step driving hormone biosynthesis within the mitochondrial matrix [19]. A similarly fascinating role of mitochondria in the secretion of catecholamines by chromaffin cells has been proposed by Montero *et al*. (2000). The authors demonstrated not only that the changes in $[Ca^{2+}]m$ during stimulation of these cells is much higher than previously estimated (they can reach values as high as 5±800 mm), but also that inhibition of mitochondrial Ca^{2+} uptake can lead to a massive increase in hormone release [25]. Similarly, long-term corticosterone (CORT) treatment also regulates the mitochondrial calcium binding capacity.

D. *The effect of sex hormones on mitochondrial calcium handling*:

The cross-talk between mitochondria and sex steroids plays a major role in the cell physiology. Indeed, sex steroids influence numerous functions of mitochondria: energy production, oxidative stress regulation, calcium homeostasis, cell proliferation or apoptosis [36], [8]. The effect of estradiol, progesterone, and testosterone on calcium ion transport in the rabbit uterus was provided by Sochorova (1990), where they investigate the effect of sex hormones on voltage-dependent calcium channels [39]. The obtained results suggest an important involvement of calcium ions in the mechanism of action of sex hormones upon the myometrial smooth muscle [39]. In contrast, it has been reported earlier that treatment with testosterone or progesterone is ineffective in altering mitochondrial calcium binding *in vitro* [19]. Likewise, estrogens have both direct and indirect beneficial effects on mitochondria that serve to preserve function under pathogenic circumstances has been reported [38]. Similarly, it has been proposed that external calcium has access to sperm only via the mitochondria and that this mitochondrial calcium is subsequently redistributed into the cytoplasmic space as a function of the internal pH [41]. The study also reported that changes in mitochondrial calcium handling properties are important in epididymal sperm maturation and suggest that the acquisition of sperm motility in the epididymis could be related to these changes in sperm calcium handling properties [41].

V. MITOCHONDRIAL CALCIUM HOMEOSTASIS AS POTENTIAL TARGET FOR MITOCHONDRIAL MEDICINE

Mitochondria are crucial in different intracellular pathways of signal transduction [9]. The alteration of the Ca^{2+} signals that reach mitochondria in association with different pathological conditions (*e.g.*, oxidative stress) can induce a profound alteration of organelle structure and function. As a consequence, the cell may be driven to its death [10]. In short, it is evident that the fine modulation of mitochondrial calcium (Ca^{2+}) homeostasis plays a fundamental role in many of the processes involving this organelle. When mitochondrial Ca^{2+}homeostasis is compromised, different pathological conditions can occur, depending on the cell type involved [9]. The study recognizes a completely different role for mitochondrial Ca^{2+} uptake. It has been well documented that insufficient mitochondrial calcium ion (Ca^{2+}) uptake or Ca^{2+} overload can cause various human diseases, such as ischemia/reperfusion injury and neurodegeneration [4], [43]. A major motivation to intervene in mitochondrial Ca^{2+} handling is to protect against ischemia/reperfusion injury, where restoring blood supply to ischemic tissues in diseases such as stroke, myocardial infarction, and peripheral vascular disease causes additional damage [42]. The mitochondrial cell death pathway which occurs through Ca^{2+}-triggered opening of mPTP, leading to the collapse of mitochondrial membrane potential and release of cytochrome C is proposed to be an important mechanism of reperfusion injury [42]. Preclinical studies have repeatedly shown the pathological roles of mitochondrial Ca^{2+} overload and the effectiveness of MCU blockade by small molecules, such as ruthenium red and Ru360, on ischemia/reperfusion injury [42]. Although Ca^{2+}transport across the inner mitochondrial membrane has been known for decades, the exact protein identities of the channels or transporters and its involvement in different pathological conditions definitely opens a new era of mitochondrial medicine.

VI. CONCLUSION:

Research over the last decade has extended the prevailing view of cell mitochondrial function well beyond its critical bioenergetic role in supplying ATP. While there is abundant information concerning its integral role in calcium homeostasis, mitochondrial calcium signaling also plays a fundamental role in inter-organellar cross-talk and thus the responses of cells to various physiological processes which remain relatively unexplored. The present review analyse some aspects of mitochondrial calcium

transport and, however a lot of research is necessary which may help in the future design of new strategies for intracellular calcium homeostasis and beyond.

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