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Review

The regulatory role of mitochondria in capacitative calcium entry

Jerzy Duszyński, Rafał Kozieł, Wojciech Brutkowski, Joanna Szczepanowska, Krzysztof Zabłocki *

Nencki Institute of Experimental Biology, 3 Pasteur Str., 02-093 Warsaw, Poland

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Abstract

Capacitative regulation of calcium entry is a major mechanism of Ca^{2+} influx into electrically non-excitable cells, but it also operates in some excitable ones. It participates in the refilling of intracellular calcium stores and in the generation of Ca^{2+} signals in excited cells. The mechanism which couples depletion of intracellular calcium stores located in the endoplasmic reticulum with opening of store-operated calcium channels in the plasma membrane is not clearly understood. Mitochondria located in close proximity to Ca^{2+} channels are exposed to high Ca^{2+} concentration, and therefore, they are able to accumulate this cation effectively. This decreases local Ca^{2+} concentration and thereby affects calcium-dependent processes, such as depletion and refilling of the intracellular calcium stores and opening of the store-operated channels. Finally, mitochondria modulate the intensity and the duration of calcium signals induced by extracellular stimuli. Ca^{2+} uptake by mitochondria requires these organelles to be in the energized state. On the other hand, Ca^{2+} flux into mitochondria stimulates energy metabolism. To sum up, mitochondria couple cellular metabolism with calcium homeostasis and signaling.

Keywords: Store-operated channel; Mitochondria; Capacitative calcium entry; Endoplasmic reticulum; Plasma membrane

1. Introduction

Ca^{2+} is one of the most important elements in cellular signaling. It participates in numerous cellular processes in all cell types, at all levels of cell differentiation and on all branches of the phylogenetic tree. In fact, it would be difficult to find a cellular process entirely independent of Ca^{2+}. It is clear therefore that intracellular calcium concentration as well as the kinetics of its changes must be precisely controlled [1].

Excitation of a cell by an extracellular stimulus generates a transient increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$). Ca^{2+} found in the cytosol comes from two sources: from the endoplasmic reticulum (ER), which is the major intracellular calcium store, and/or from the extracellular space. The difference between $[Ca^{2+}]$ of the cytosol and the lumen of ER or the extracellular fluid reaches 3 and 4 orders of magnitude, respectively. Thus, opening of calcium channels in the ER membranes or in the plasma membrane (PM) is sufficient to allow fast passive flux of Ca^{2+} into the cytosol. Therefore, opening of the calcium channels must be rigorously controlled. Closure of Ca^{2+} channels and removal of cytosolic Ca^{2+} terminate the calcium signal. The latter process is energy consuming. Ca^{2+}extrusion from the cytosol occurs at the expense of ATP and/or Na^+ gradient across PM and requires participation of Ca^{2+}-ATPases located in both membranes (ER and PM) and/or involvement of a Na^+/Ca^{2+} exchanger in PM [2,3].

Various types of Ca^{2+} channels exist within the PM. Among them are voltage-gated channels (typical for electrically excitable cells), ligand-gated channels, channels activated by intracellular messengers (such as arachidonic acid or calcium ions) and store-operated channels (SOCs) (for a review, see [4]). Flux of Ca^{2+} through the SOCs is also referred to as capacitative calcium entry (CCE). This process was originally described 20 years ago by Putney and co-workers, and it has generated ample literature to date [5–9]. In spite of intensive studies, CCE remains the most mysterious regulatory mechanism engaged in the opening of plasma membrane Ca^{2+} channels. Although CCE is the most common and the most important route of

* Corresponding author. Tel.: +48 22 5892497; fax: +48 22 8225342.
E-mail address: k.zablocki@nencki.gov.pl (K. Zabłocki).

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calcium flux into electrically non-excitable cells, much evidence suggests that it also plays a role in excitable ones (i.e., neurons or skeletal muscle cells) [10–12]. According to this mechanism calcium flux into the cell is controlled by the filling state of intracellular calcium stores [13].

Two important functions of CCE have been recognized so far: (i) replenishment of intracellular calcium stores partially depleted due to agonist stimulation, (ii) enhancement of calcium signals and extension of their duration when it is necessary for physiological reasons (for example, during activation of lymphocytes having particularly small ER calcium stores) and modification of Ca^{2+} oscillations.

Stimulation of cells leading to activation of CCE followed by reversal of the resting state comprises three major steps: (i) agonist-induced depletion of calcium stores, (ii) opening of SOCs and Ca^{2+} influx, (iii) increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) followed by the refilling of ER stores and eventually closure of SOCs. The dynamic changes in the free cytosolic $[Ca^{2+}]$ and in the lumen of ER are controlled by a number of factors including Ca^{2+} transporting systems (Ca^{2+} channels and Ca^{2+}-ATPases in ER and PM and Ca^{2+}/Na^{+} exchanger in PM, both counteracting the former) and intracellular calcium buffers (calcium binding proteins, nucleotides and phosphate). Moreover, mitochondria have been postulated to play an important role as calcium buffers participating in the intracellular calcium handling machinery in stimulated cells [14–16]. Recently, mitochondria have been considered not only as high-capacity Ca^{2+} buffers but also as "active" elements of the intracellular signaling system [17,18].

2. Mitochondrial calcium handling

Mitochondria participate in the modulation of Ca^{2+} signals in many cell types including both electrically non-excitable and electrically excitable ones. This mitochondrial function is based on their ability to uptake calcium and accumulate it in the matrix, which in turn is tightly coupled to their energy state. Ca^{2+} enters the mitochondrial matrix through a uniporter located in the inner membrane. The process is driven by mitochondrial potential ($\Delta\Psi$) and is sensitive to ruthenium red. On the other hand, increase in the intramitochondrial Ca^{2+} concentration within the physiological range enhances mitochondrial catabolic processes due to stimulation of three mitochondrial dehydrogenases: pyruvate-, isocitrate- and 2-oxoglutarate- [19]. Therefore, mitochondrial metabolism and calcium homeostasis are mutually connected. The Ca^{2+}-activated metabolism supports generation of mitochondrial potential which in turn is necessary for the calcium influx to occur.

Experiments in vitro have shown that the affinity of the mitochondrial uniporter for Ca^{2+} is relatively low (K_d seems to be as high as 10 μM). Since global $[Ca^{2+}]_c$ amounts to 100 nM and 1 μM in the resting and activated cells, respectively, one could conclude that mitochondria in situ are not able to take up calcium effectively. Indeed, it is accepted that calcium accumulation in the mitochondrial matrix starts when extramitochondrial $[Ca^{2+}]$ exceeds 400 nM. This results from the balance between the low-affinity calcium uptake and the efficient calcium extrusion from the mitochondrial matrix, occurring via Ca^{2+}/Na^{+} or Ca^{2+}/H^{+} antiporters ([20] and references therein). The apparent discrepancy between the well-documented calcium entry into mitochondria in situ and the high K_d of the Ca^{2+} uniporter preventing this process has been explained with a discovery that the local concentration of $[Ca^{2+}]$, especially in the vicinity of ER and PM calcium channels, may exceed many times the bulk cytosolic calcium concentration. Mitochondria located in those subcompartments are exposed to high $[Ca^{2+}]$, sufficient for an effective calcium uptake and accumulation in the mitochondrial matrix. Therefore, only mitochondria located in close vicinity of various calcium channels directly play a role in the shaping of the intracellular calcium signals. This explanation came from experiments engaging advanced techniques for specific visualization of organelles and for determination of local Ca^{2+} concentration. Such tests became possible only after methods based on transfection of cells with calcium-sensitive fluorescent proteins targeted to selected compartments had been developed [21–27].

At present, it is well documented that mitochondria undergo dynamic fusion and fission leading to the formation of a mitochondrial network or to its fragmentation into discrete organelles dispersed throughout the cell [28,29]. Such dynamic balance between the two states of mitochondrial organization depends on cell type and their physiological/metabolic status. A decrease in mitochondrial potential inevitably causes fragmentation of the mitochondrial network. These findings explain the special function of mitochondria in the regulation of calcium signals and in the maintenance of calcium homeostasis. Juxtaposition of PM Ca^{2+} channels and mitochondria as well as close contacts between mitochondria and IP_3-gated calcium channels in ER allow mitochondria to accumulate Ca^{2+}. This accumulation decreases local $[Ca^{2+}]$ and thereby affects calcium-dependent processes. Moreover, the extensive mitochondrial network may connect various intracellular microcompartments, thus enabling intramitochondrial calcium tunneling [21].

3. Mitochondria regulate calcium release from ER

Calcium release from ER stores requires opening of Ca^{2+} channels in ER membranes. However, the efficiency of depletion of calcium stores depends not only on the rate of Ca^{2+} release but also on the counteracting activity of SERCA (Sarco/Endoplasmic Reticulum Calcium ATP-ase) which drives the refilling of the Ca^{2+} stores. The mode of activation of calcium release from ER stores depends on the type of agonist used for cell excitation. When it acts via one of the isoforms of phospholipase C, an increase in IP_3 concentration triggers the opening of the IP_3-receptor-coupled calcium channels in the ER membrane. The efficiency of IP_3 action also depends on $[Ca^{2+}]_c$ which may exert a concentration-dependent positive or negative feedback effect on the Ca^{2+} channels. Some IP_3 receptors (IP_3R-1) exhibit a bell-shaped curve of dependency on $[Ca^{2+}]$ [30]. Thus, modulation of the

local Ca^{2+} concentration may influence the opening probability of calcium channels and therefore may affect the effectiveness of calcium release [31].

Opening of calcium channels within the ER membrane may also be induced by a transient primary increase in $[Ca^{2+}]_c$ resulting from Ca^{2+} influx into the cell via agonist-sensitive PM calcium channels (ligand-gated channels) or membrane potential-sensitive channels (voltage-gated calcium channels typical for electrically excitable cells). This phenomenon, known as Calcium Induced Calcium Release (CICR), engages ryanodine receptors/calcium channels within the ER or SR (for sarcoplasmic reticulum, in a case of muscle cells) membranes, which are additionally modulated by cADP-ribose [1]. In other words, calcium release from ER/SR stores is strongly influenced by locally accumulated Ca^{2+} coming from either ER or extracellular space. It is noteworthy that both modes of the intracellular Ca^{2+} store depletion, either through IP_3-dependent channels as described for electrically non-excitable cells [7–9] or through RyRs as described for mouse skeletal muscle fibres [11] and for cultured myotubes [32], may participate in the activation of CCE. Interestingly, during long-lasting stimulation of Jurkat cells (typical electrically non-excitable ones) primary depletion of ER stores through IP_3-activated channels seems to be replaced by calcium release via RYRs. This activates the loop between capacitative calcium entry and CICR which allows to maintain sustained calcium signal over the period of minutes or even hours [33].

Mitochondria located in close vicinity of ER, especially those neighboring calcium channels, are exposed to a relatively high $[Ca^{2+}]_c$ which flows from calcium stores to the cytosol. As a result, they are able to act as effective, dynamic Ca^{2+} buffers decreasing local $[Ca^{2+}]$. Accordingly, they modify the opening probability of Ca^{2+} channels in the ER membranes increasing the dynamic range of $[IP_3]$ over which it is able to trigger CCE in Jurkat cells. In addition, they compete with SERCA for Ca^{2+}, thus supporting a depletion of ER stores and delaying the slow inactivation of capacitative calcium entry that follows replenishment of calcium stores [34]. Close contacts between mitochondria and ER membranes, as described in HeLa cells [26], significantly increase the efficiency of mitochondrial accumulation of Ca^{2+} released from the stores, which promotes an activation of CCE. In conclusion, mitochondria-dependent changes in the Ca^{2+} concentration in the proximity of IP_3 receptors may modulate (enhance or reduce) the opening of ER Ca^{2+} channels (Fig. 1). This in turn affects calcium release and shapes the calcium signal. In other words, mitochondria protect these receptors against feedback inhibition when the free Ca^{2+} concentration exceeds the optimal value. The exact effect of mitochondria on this process depends on the receptor type and the real $[Ca^{2+}]_c$ in a given subcompartment. Similarly, because the activity of ryanodine receptors strongly depends on the local $[Ca^{2+}]_c$, it is clear that mitochondria may also modulate calcium release via RYRs, which in turn may affect CCE, as described for rabbit pulmonary arterial smooth muscle cells [35].

It ought to be stressed here that the role of such cross-talk between mitochondria and calcium stores is not limited to the regulation of ER depletion preceding the activation of capacitative calcium entry into cells. Activation of a cell may also lead to partial depletion of ER calcium stores and generation of intracellular calcium signals known as calcium waves and oscillations. In such case, stimulation of Ca^{2+} entry into cells may not be obligatory. Subtle release of Ca^{2+} through a very limited number of Ca^{2+} channels (so-called Ca^{2+} sparks) may sensitize neighboring Ca^{2+} receptors/channels and eventually support Ca^{2+} release along the ER network. This generates calcium waves which propagate throughout the cell. Buffering of Ca^{2+} by mitochondria in close vicinity to ER Ca^{2+} channels plays a role in the modulation of these elementary Ca^{2+} events (calcium sparks), spreading of Ca^{2+} waves and generation of calcium oscillations within the cell. On the other hand, accumulation of a certain amount of Ca^{2+} by mitochondria activates oxidative

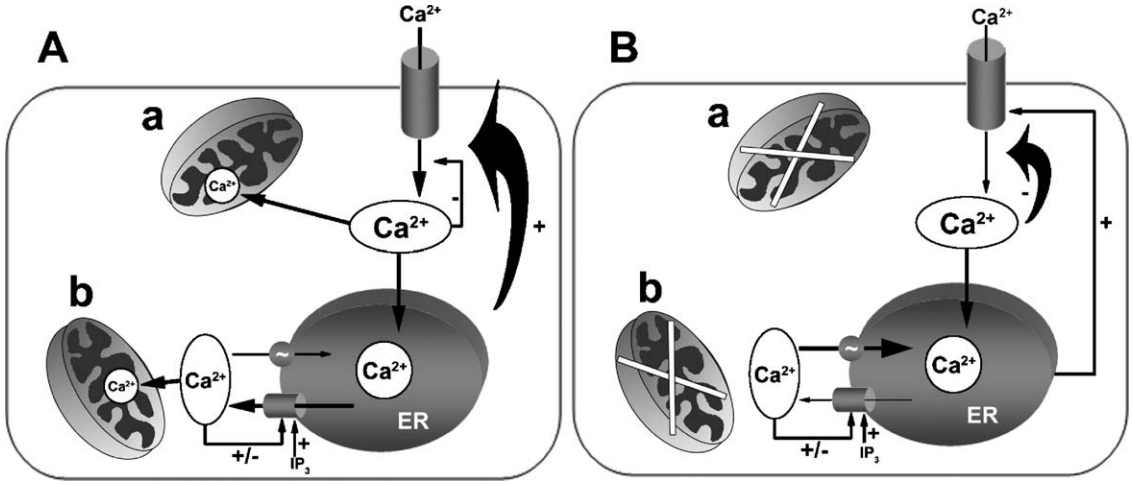

Fig. 1. Mitochondria regulate capacitative calcium entry into electrically non-excitable cells. (A) Energized mitochondria support Ca^{2+} influx. (a) Mitochondria located in close vicinity to PM protect SOC from feedback inhibition. (b) Mitochondria in close contact with ER compete with Ca^{2+}-ATPase for Ca^{2+} and modulate Ca^{2+} release through IP_3-activated channels. Thus, they increase the efficiency of depletion of ER stores. (B) Deenergized (uncoupled) mitochondria are not able to buffer Ca^{2+}. (a) Mitochondria located in close vicinity to PM do not protect SOC from feedback inhibition (store-independent slow inactivation of CCE). (b) Mitochondria in close contact with ER do not counteract ER replenishment (store-dependent slow inactivation of CCE).

phosphorylation. The exact effect of such "collaboration" between mitochondria and ER strongly depends on the cell type (for reviews, see [1,4]), as shown by the following examples: (i) close structural and functional contacts between mitochondria and endo/sarcoplasmic membranes are responsible for the increase in mitochondrial calcium concentration and for generation of intramitochondrial Ca^{2+} oscillations in depolarized C2C12 myotubes [36]. This mechanism may coordinate depolarization-evoked contraction of cells with Ca^{2+}-dependent stimulation of mitochondrial energy metabolism. (ii) In permeabilized mammalian skeletal muscle fibres energized mitochondria located in close vicinity to sarcoplasmic reticulum prevent calcium sparks. In these cells, Ca^{2+} sparks (also called "elementary events of CICR") result from the activation of ryanodine receptors in SR. Deprivation of mitochondria of respiratory fuel or inhibition of the respiratory chain, dissipation of mitochondrial potential by protonophores or inhibition of mitochondrial Ca^{2+} uniporter block mitochondrial Ca^{2+} accumulation and promote generation of Ca^{2+} sparks [37]. Finally, it was concluded that under physiological conditions mitochondria actively suppress spontaneous Ca^{2+} sparks in mammalian muscle fibres. It was also postulated that appearance of spontaneous Ca^{2+} sparks was dependent on the mitochondrial redox state. Increased production of reactive oxygen species (ROS) promotes the generation of Ca^{2+} sparks while effective scavenging of ROS prevents them [38]. (iii) Periodic spiking of cytosolic Ca^{2+} concentration (calcium oscillations) observed in many activated cell types and recently described for HeLa cells is attributed, at least to some extent, to repetitive Ca^{2+} shuttling between ER and mitochondria [39].

In many cell types, mitochondria limit propagation of calcium waves or confine the calcium signal to a restricted intracellular region. In ATP-activated rat cortical astrocytes mitochondrial depolarization enhances propagation of Ca^{2+} waves throughout the cells [40]. In pancreatic acinar cells stimulated to secrete, energized mitochondria are necessary for calcium signal to be confined to the secretory pole. Deenergization of mitochondria in these cells leads to abnormal spreading of calcium signals throughout the cell. Notably, within these highly specialized cells, a large portion of mitochondria surround the apical pole (perigranular mitochondria), forming a "firewall" protecting the cells against uncontrolled diffusion of Ca^{2+}. Two other portions of mitochondria in pancreatic acini are distributed in the subplasmalemmal and perinuclear subcompartments. This pattern of intracellular distribution of mitochondria supports the hypothesis of functional heterogeneity among these organelles [23]. On the other hand, in some cases, for example in *Xenopus* oocytes, energization of mitochondria facilitates spreading of calcium signal throughout the cell [41].

4. Mitochondria regulate capacitative calcium entry into cells

Contrary to the notion of a very close if not even structural interaction between ER membranes and mitochondria, there is no evidence indicating such "intimate" contacts between mitochondria and PM. However, a growing body of evidence indicates that mitochondria located in the subplasmalemmal space have a special function as Ca^{2+} buffers which locally decrease cytosolic calcium concentration and therefore affect Ca^{2+}-dependent processes in this area [42].

Store-operated channels create a family of cation channels of different specificities and electrophysiological properties. Some of them are sensitive to intracellular Ca^{2+}; thus, they may undergo self-regulation by a negative feedback response (for a review, see [43]). Lymphocytes and some cell lines such as RBL (Rat Basophilic Leukaemia) and human lymphoidal (Jurkat) cells attract special attention because they express a calcium channel which is the "golden standard" for the SOC family. In these cells, depletion of ER calcium stores activates Ca^{2+} channels of high specificity for Ca^{2+} and very low conductance. Ca^{2+} flow through this channel is known as J_{CRAC} (Calcium Released Activated Current) [8,44–47]. Feedback inhibition of J_{CRAC} by Ca^{2+} accumulated in subplasmalemmal space is, together with the progressive refilling of ER stores, responsible for slow inactivation of CCE [48]. Energized mitochondria accumulate the Ca^{2+} entering the cells. This protects SOCs against feedback inhibition and prolongs CCE [14,49]. Uncouplers of oxidative phosphorylation or inhibitors of the respiratory chain abolish mitochondrial membrane potential and therefore block mitochondrial calcium uptake, which in turn inhibits capacitative calcium influx (Fig. 1). Interestingly, a similar effect on CCE may be achieved by inhibition of mitochondrial Ca^{2+}/Na^{+} exchanger, confirming the hypothesis of intramitochondrial calcium tunneling from the subplasmalemmal space to ER [50]. This phenomenon may be required for efficient reloading of intracellular calcium stores. Inhibition of Ca^{2+} release from mitochondria leads to their overloading, which stops mitochondrial Ca^{2+} uptake, inhibits calcium entry into the cell and prevents store replenishment [21].

The impact of mitochondrial calcium buffering on the regulation of CCE is not a fixed feature of a given cell type. For example, changes in extracellular pH may significantly alter the relations between mitochondrial Ca^{2+} uptake and the rate of CCE in Jurkat cells (Fig. 2). If extracellular pH is lower than 7.4 dissipation of mitochondrial potential or inhibition of mitochondrial Na^{+}/Ca^{2+} exchanger significantly reduce Ca^{2+} entry. Alkalinization of the extracellular milieu to pH 7.8 makes CCE less sensitive to mitochondrial inhibitors. It may be concluded that the Ca^{2+} channel may somehow sense the extracellular pH and switch its conformation between one very sensitive to intracellular Ca^{2+} (at $pH<7.4$) and one relatively insensitive (at $pH>7.4$) [50]. Alternatively, it might be speculated that changes in extracellular pH which modify H^{+} current through voltage-activated proton channels [51,52] affect intracellular pH and therefore influence mitochondrial energy metabolism and calcium buffering capacity, which in turn could enhance slow inactivation of calcium influx. However, such explanation may be excluded for three reasons: (i) in Jurkat cells suspended in the buffer of pH 7.2 supplemented with 3 mM $CaCl_2$ and pretreated with CCCP for uncoupling of oxidative phosphorylation and with thapsigargin for activation of CCE, selective alkalinization of the cytosol by the addition of either ammonium

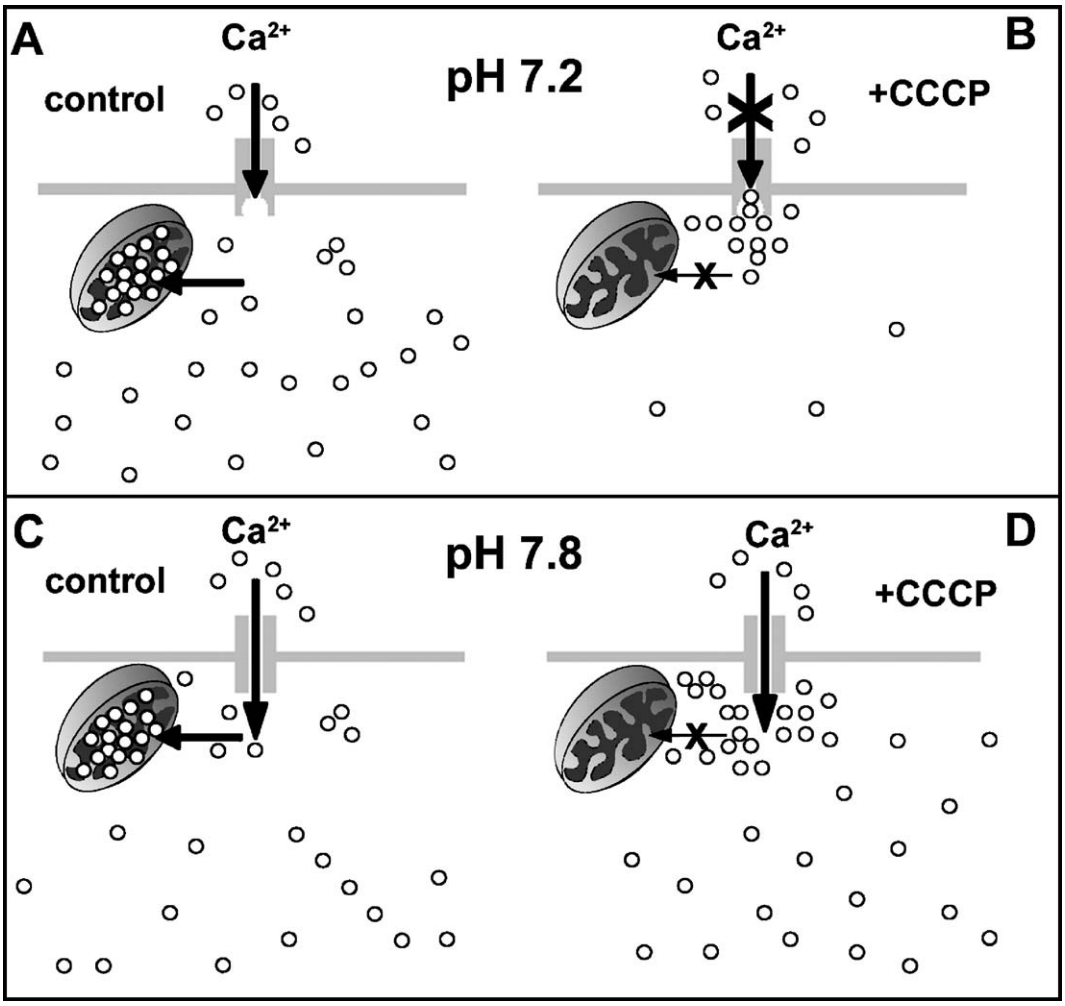

Fig. 2. In Jurkat cells extracellular pH modifies SOC sensitivity to intracellular Ca^{2+}. Cells suspended in pH 7.2 buffer. (A) Energized mitochondria protect SOC from feedback inhibition. Intensive Ca^{2+} entry. (B) Deenergized mitochondria do not buffer Ca^{2+}. Ca^{2+} entry is reduced due to feedback inhibition. Cells suspended in pH 7.8 buffer. (C) Energized mitochondria buffer Ca^{2+}. Intensive Ca^{2+} entry. (D) Deenergization of mitochondria does not affect Ca^{2+} influx. In Jurkat cells SOC exists in two conformations: sensitive and insensitive to intracellular Ca^{2+}. Transition between them depends on the extracellular pH. At a lower pH the Ca^{2+}-sensitive form (mitochondrial Ca^{2+} buffering required) dominates while under more alkaline conditions the Ca^{2+}-insensitive form prevails (figure prepared on the basis of [50]).

chloride or methylamine does not affect steady-state level of cytosolic Ca^{2+} concentration [49,50]; (ii) mitochondrial uncoupler (CCCP) dissipates $\Delta\Psi$ independently of the extracellular pH while its inhibitory effect on Ca^{2+} entry was visible only in cells suspended in the buffers of pH>7.4 [49,50]; (iii) depolarization of plasma membrane (due to increase in KCl concentration in the bath solution or by inhibition of K^+,Na^+-ATPase by oubain), although partially inhibits the rate of capacitative calcium entry does not influence its sensitivity to mitochondrial uncouplers (Zablocki, unpublished data).

Stimulatory effects of mitochondria on CCE, based on the protection of SOCs against feedback inhibition, have been described in Jurkat cells, RBL cells, and human T lymphocytes [14]. More recently, the role of mitochondria as a Ca^{2+} buffers preventing slow inactivation of SOC was described in cultured myotubes [32].

In activated lymphocytes in which a long-lasting Ca^{2+} signal forms Ca^{2+} oscillations of precisely regulated frequency and amplitude, CCE is the major route for Ca^{2+} entry. These cyclic changes in $[Ca^{2+}]_c$ determine cellular response to excitation, expressed as the synthesis of appropriate transcription factors. Modifications of the calcium signaling profile may significantly affect the pattern of protein synthesis. Mitochondria acting as Ca^{2+} buffers and thereby regulators of CCE are, together with other intracellular tools (ER stores, Ca^{2+}-binding proteins, Ca^{2+} pumps and transporters), important participants involved in the modulation of Ca^{2+} oscillations [53].

Mitochondrial regulation of CCE is not however universal among all cell types. For example, in human osteosarcoma cells, mitochondrial uncoupling does not affect the rate of store-operated calcium influx. Instead, it enhances the amplitude of Ca^{2+} transient, indicating that mitochondrial buffering of Ca^{2+} does not support Ca^{2+} entry but decreases the intensity of calcium signal. It seems possible that calcium channels activated due to depletion of the ER calcium stores in these cells are insensitive to intracellular Ca^{2+} [54]. In such cases, deprivation of mitochondria of their capacity to accumulate Ca^{2+} leads to an abnormal increase in cytosolic Ca^{2+} concentration. Participation of mitochondria in CCE was also studied in HeLa cells with

experimentally impaired mitochondrial structure and intracellular localization [55]. Relocalization of mitochondria from the subplasmalemmal space towards the nucleus due to expression of dynamitin significantly decreased CCE into the cells confirming the role of mitochondria as calcium buffers preventing feedback inhibition of calcium channels [55]. Opposite results were obtained for HeLa cells transfected with hFis1. Expression of this gene results in fragmentation (fission) of the mitochondrial network into discrete organelles which shift from the subplasmalemmal space to a deeper located perinuclear region. Surprisingly, these changes in intracellular mitochondrial organization only slightly decreased histamine-induced Ca^{2+} entry, while mitochondrial uncoupling in such cells seriously inhibited Ca^{2+} influx. The reason of such discrepancy needs to be elucidated. However, the poor correlation between relocalization of mitochondria in hFis1-overexpressing cells and the intensity of Ca^{2+} entry together with the evident dependency of calcium influx on $\Delta\Psi$ might indicate that, at least in HeLa cells, mitochondria do not act only as passive Ca^{2+} buffers preventing feedback inhibition of SOCs. It was hypothesized that energized mitochondria release a putative messenger which activates Ca^{2+} entry [56]. A similar possibility was also considered for RBL cells [57]. It seems likely that mitochondria may have a double function in the regulation of Ca^{2+} entry: a "passive" (as Ca^{2+} buffers) and an "active" one (as a source of putative messenger molecule), but participation in the two roles may differ for various cell types. The role of mitochondria as a source of a diffusible messenger which promotes calcium entry into stimulated cells was also hypothesized by Ayub and Hallett [58], who indicated adenosine diphosphate ribose (ADPR) as a putative mitochondrially derived molecule activating SOCs in immune cells. According to them uptake of Ca^{2+} (released from ER stores due to stimulation of cells) by mitochondria tightly connected with ER network is a prerequisite for synthesis of ADPR from NAD^+. ADPR generated in the mitochondrial intermembrane space is postulated to diffuse to the plasma membrane and activate Ca^{2+} entry into cells. The necessity of mitochondrial Ca^{2+} uptake for activation of ADPR formation in/release from mitochondria explains the sensitivity of CCE to mitochondrial uncouplers.

It must be also pointed out that SOCs are not the only channels exhibiting feedback inhibition by Ca^{2+} accumulated in the subplasmalemmal space. For example, in rat cortical brain slices stimulated by glutamate, inhibition of mitochondrial Ca^{2+} uptake strongly reduces Ca^{2+} entry through the NMDA ionotropic receptor [59].

Mitochondrial accumulation of Ca^{2+} entering cells and consequential changes in $[Ca^{2+}]_c$ in the subplasmalemmal space not only affect calcium influx per se but also strongly affects the opening of the Ca^{2+}-activated potassium channel (BK channel) in PM. Increase in the local Ca^{2+} concentration in close vicinity to BK channel activates K^+ flux whereas decrease in Ca^{2+} concentration inhibits flow of K^+ ions. Stimulation of K^+ efflux from the cells increases polarization of PM. Thus, regulation of local cytosolic Ca^{2+} by mitochondria efficiently regulates K^+ current and therefore affects polarization of the plasma membrane [42]. Because CCE is supported by the electric potential across PM, Ca^{2+}-evoked activation of BK channels accelerates Ca^{2+} entry into the cell. In this way, the store-operated Ca^{2+} channel and Ca^{2+}-dependent K^+ channel, both located within the PM, cooperate in the positive feedback loop during Ca^{2+} entry in activated cells, i.e., in activated lymphocytes [53].

5. Mitochondrial pathology and calcium signal

Around 90% of ATP produced in aerobic cells comes from oxidative phosphorylation. Therefore, any impairment of mitochondrial metabolism may affect the energy state of the whole cell. This happens in the presence of mitochondrial toxins, as a result of damage to mitochondrial DNA or as a consequence of mitochondrial genetic disorders. Diminished ATP production decreasing cellular ATP concentration a below critical point reduces the activity of Ca^{2+}-ATPases, which in turn results in abnormally increased $[Ca^{2+}]_c$ and prolonged duration of Ca^{2+} signals. These latter effects induce several changes in the cellular metabolism and function including activation of Ca^{2+}-dependent enzymes (calpains, PKC) and induction of mitochondrial permeability transition pore (PTP), which may eventually lead to apoptosis. Pathological symptoms related to ATP deficiency are especially visible in tissues of high-energy demand (heart, skeletal muscle, central nervous system) [60]. Mitochondrial participation in the processes of aging may also be partially explained by insufficient ATP production resulting from age-related oxidative damage of mitochondria [61].

Apart from the direct consequences of cellular ATP deficiency, a decrease in mitochondrial potential reduces Ca^{2+} uptake by these organelles, which may be an additional factor significantly influencing Ca^{2+} homeostasis and cellular calcium signals. In human fibroblasts isolated from patients suffering from Leigh disease caused by SURF-1 mutation, Ca^{2+} influx following the depletion of ER stores is significantly reduced [62].

Mitochondrial Ca^{2+} uptake is beneficial for energy metabolism, unless cytosolic Ca^{2+} concentration exceeds the physiological range. If it happens mitochondria accumulate too much calcium (calcium overload) which in turn damages mitochondrial functioning and supports cell death. This scenario is proposed for glutamate excitotoxicity in neurons ([63] and references therein), post-ischemic damage of neurons (for a review, see [64]) and heart muscle cells [65], and Duchenne dystrophy-related cell damage [66]. Recently, it has been shown that enhanced CCE observed in the dystrophin-deficient cultured myotubes leads to the excessive mitochondrial Ca^{2+} accumulation, whereas reintroduction of minidystrophin into such cells prevents accelerated CCE which in turn limits mitochondrial Ca^{2+} uptake [32]. This observation additionally confirms the link between abnormal Ca^{2+} entry, mitochondrial Ca^{2+} overloading and dystrophy-induced pathology.

6. Conclusions

In conclusion, accumulation of Ca^{2+} by mitochondria may have complex effects on cellular Ca^{2+} homeostasis: (i) mitochondria buffer locally the excess of cytosolic Ca^{2+} thereby increasing Ca^{2+} clearance and decreasing the amplitude of calcium transients [67]. Moreover, after termination of cell excitation and subsequent decrease in $[Ca^{2+}]_c$ mitochondria release the accumulated calcium, which prolongs the phase of enhanced $[Ca^{2+}]_c$ [68]; (ii) mitochondria prevent slow inactivation of CCE by buffering Ca^{2+} close to Ca^{2+} channels in both ER and PM [16,34,48]; (iii) mitochondria support the reloading of ER calcium stores, which in turn may limit CCE [69]. The overall effect of mitochondrial action depends most probably on the subcellular spatial organization of the mitochondrial and ER networks and on the distances between appropriate subdomains of these structures. This may be related to the cell type and the mechanism of cell stimulation. Finally, mitochondrial Ca^{2+} accumulation is tightly connected with their energy status. This links cellular energy metabolism with the spatiotemporal shaping of calcium signals.

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