

Ca²⁺ signalling in mitochondria: mechanism and role in physiology and pathology

Marisa Brini^{a,b,*}

^a Department of Biochemistry and Center for the Study of Biomembranes of the National Research Council (CNR), University of Padova, Viale G. Colombo 3, Padova 35121, Italy

^b Venetian Institute of Molecular Medicine (VIMM), Via Orus 2, 35129 Padova, Italy

Received 10 May 2003; received in revised form 12 May 2003

Abstract

Over recent years, a renewed interest in mitochondria in the field of Ca²⁺ signalling has highlighted their central role in regulating important physiological and pathological events in animal cells. Mitochondria take up calcium through an uptake pathway that, due to its low-Ca²⁺ affinity, demands high local calcium concentrations to work. In different cell systems high-Ca²⁺ concentration microdomains are generated, upon cell stimulation, in proximity of either plasma membrane or sarco/endoplasmic reticulum Ca²⁺ channels. Mitochondrial Ca²⁺ accumulation has a dual role, an universal one, which consists in satisfying energy demands by increasing the ATP production through the activation of mitochondrial enzymes, and a cell type specific one, which, through the modulation of the spatio-temporal dynamics of calcium signals, contributes to modulate specific cell functions. Recent work has revealed the central role of mitochondria dysfunction in determining both necrotic and apoptotic cell death. Evidence is also accumulating that suggests that alterations in mitochondrial function may act as predisposing factors in the pathogenesis of a number of neurodegenerative disorders. These include inherited disorders of the mitochondrial genome in which a defect in mitochondrial calcium accumulation has been shown to correlate with a defect in ATP production, thus suggesting a possible involvement of mitochondrial Ca²⁺ dysfunction also for this group of diseases. This review analyses recent developments in the area of mitochondrial Ca²⁺ signalling and attempts to summarise cell physiology and cell pathology aspects of the mitochondrial Ca²⁺ transport machinery.

© 2003 Elsevier Ltd. All rights reserved.

1. Introduction

Calcium plays a central role in cellular signalling [1–3]. Its concentration in the cellular environment changes in response to a variety of signals, which differ in their origin (extracellular or intracellular), and in their chemical, mechanical or electrical nature. Like other signalling ions or molecules, generally an increase in its cytosolic concentration corresponds to an activation of a cellular function. The specificity of the activated function depends on several factors: on the co-ordinate activity of the Ca²⁺ transport and the Ca²⁺-binding proteins, as well as on the intracellular compartmentalisation, represented by the organelles, which contributes to generate the well-described spatial and temporal complexity of Ca²⁺ signal. Until recently, organelles were considered not only physically but also functionally distinct in respect to the control of Ca²⁺ homeostasis. In particu-

lar, the endoplasmic reticulum was considered the dynamic Ca²⁺ regulator in the cell and, at variance, the nucleus, the mitochondria and the Golgi compartment were relegated only to a marginal role. The recent development of molecular biology and cell imaging techniques has revealed that, in living cells, all these components are strictly interconnected and the control of their homeostasis not only is essential in the control of organelles specific processes but also is fundamental in the dynamic modulation of the cytosolic Ca²⁺ signalling.

In this review I will focus on mitochondrial Ca²⁺ homeostasis. These organelles were considered to play a major role in intracellular Ca²⁺ homeostasis at the beginning of the 1960s when it was discovered that they could rapidly take up large calcium loads (suggesting that mitochondria probably acted as large reservoir of Ca²⁺). Then, they were relegated to oblivion for 30 years, after it was observed that the affinity of mitochondrial Ca²⁺ transporters was too low to allow significant uptake at the cytosolic Ca²⁺ concentration of resting (~0.1 μM) and stimulated (~1–3 μM) cells. At the present mitochondria and their Ca²⁺ homeostasis have

* Tel.: +39-049-8276167; fax: +39-049-8276125.

E-mail address: marisa.brini@unipd.it (M. Brini).

been reevaluated: it was demonstrated that not only mitochondria can efficiently take up Ca^{2+} at physiological cytosolic Ca^{2+} concentration but that mitochondrial Ca^{2+} accumulation strictly controls the energetic metabolism of the cells, by directly modulating the activity of enzymes located in the matrix, and contributes to shaping cytosolic Ca^{2+} fluctuations, thus in turn modulating cellular functions regulated by cytosolic Ca^{2+} variations.

This review summarizes our current knowledge of mitochondrial Ca^{2+} homeostasis considering both the physiological aspects in different cell types and the pathological consequences linked to mitochondrial Ca^{2+} signalling disturbance.

2. Control of mitochondrial calcium homeostasis

Mitochondrial Ca^{2+} transport was discovered in the early 1960s and it was originally described as active Ca^{2+} uptake and passive release. Later the chemiosmotic theory and the measurement of internally negative membrane potentials led to the concept of an energetically downhill uptake mechanism. At the beginning of these studies, mitochondria were considered the major intracellular stores of the ion. When, however, their Ca^{2+} uptake system was characterised, its affinity was found to be too low to accumulate the cation not only in resting conditions but also during the transient cytosolic Ca^{2+} concentration increases generated by cell stimulation. In the eighties, the identification of inositol 1,4,5-trisphosphate (InsP_3) as soluble second messenger that liberated Ca^{2+} from an internal store (soon identified as the endoplasmic reticulum [4]) definitely excluded the mitochondria from the role of mobilizable intracellular Ca^{2+} sinks. The idea that mitochondria could accumulate Ca^{2+} when its concentration greatly increased in the cytoplasm became prevalent and these organelles were relegated to the role of safeguards against Ca^{2+} overloads, to prevent cellular toxicity and damage. This role was thus considered the more relevant one although the work of Denton and co-workers [5,6] demonstrated that Ca^{2+} regulates the activity of three key enzymes of mitochondrial metabolism. Indeed, although it was not clear how mitochondria could accumulate Ca^{2+} in physiological conditions, these data strongly suggested that the regulation of the matrix enzymes was a possible target for Ca^{2+} rises occurring in the mitochondrial matrix, and thus that this process should occur in healthy cells.

The kinetic properties, the sensitivity to inhibitors and the mechanism of action of the mitochondrial transport systems have been extensively studied starting from the sixties (see [7] for a detailed review). It is now well recognised that the large negative potential across the inner membrane (up to -180 mV), generated by the respiratory chain, drives Ca^{2+} into mitochondria matrix through an electrogenic transporter, the uniporter, located in the inner membrane. The uniporter, which is specifically inhibited by ruthenium red (RR) and divalent cations (e.g. Sr^{2+} , Mn^{2+} ,

Ba^{2+} and lanthanides, which are themselves transported by the uniporters), tends to distribute Ca^{2+} according to its electrochemical gradient and thus promotes accumulation of the ion in the matrix. The electrochemical equilibrium is prevented by the activity of the electroneutral exchangers, which transport Ca^{2+} out of the matrix in exchange for H^+ or Na^+ . The Ca^{2+} affinity of the uniporter in isolated mitochondria was estimated to be in the range of $10 \mu\text{M}$, one or two order of magnitude higher than the Ca^{2+} concentration values of $0.1\text{--}2 \mu\text{M}$ measured in the cytoplasm of living cells with Ca^{2+} fluorescent probes. The Na^+ -independent Ca^{2+} efflux saturates at very low- Ca^{2+} loads and is relatively slow; in addition it requires a transmembrane potential as a component of its driving force. The Na^+ -dependent Ca^{2+} efflux is considered as the pathway that mediates physiological Ca^{2+} cycling. The kinetics parameters for Ca^{2+} efflux are somewhat variable in mitochondria from different sources, i.e. heart mitochondria show the maximal rate of Ca^{2+} transport. Ca^{2+} efflux is inhibited by Sr^{2+} , Ba^{2+} , Mg^{2+} , Mn^{2+} and by a variety of compounds of pharmacological interest such as amiloride, diltiazem, clonazepam and CGP37157. The molecular nature of the uniporter, as well as that of the mitochondrial Ca^{2+} export systems, the antiporters $\text{H}^+/\text{Ca}^{2+}$ and $\text{Na}^+/\text{Ca}^{2+}$, is unknown. Moreover, a putative additional pathway for Ca^{2+} release, the permeability transition pore (PTP), has been recently described. This non-selective, high-conductance channel has attracted much interest as it was proposed that its activation in early phases of the apoptotic process could cause the swelling of mitochondria, the rupture of the outer membrane and the consequent release of caspase co-factors such as cytochrome c in the cytoplasm. Despite an intense body of work, however, also the molecular nature of this channel is unknown.

The vast majority of information summarised until now comes from biochemical studies carried out on isolated mitochondria and only recently the introduction of the recombinant probe aequorin selectively targeted to mitochondrial matrix [8] and the new generation of fluorescent dyes, i.e. rhod-2, which selectively accumulate into the mitochondria [9,10], and Ca^{2+} sensitive GFPs have permitted to investigate mitochondrial Ca^{2+} homeostasis in living cells. These important technological advances have shown that mitochondria, in a variety of cell types, accumulate Ca^{2+} very efficiently and rapidly, due to the fact that their uniporter is exposed to local Ca^{2+} concentrations much higher than those measured in the bulk cytosol. In fact, the cytosolic Ca^{2+} concentration does not increase uniformly during cell stimulation, this implies that local regions of high-cytosolic Ca^{2+} are generated in close proximity of the Ca^{2+} channels of the plasma membrane which allow the Ca^{2+} entry from the extracellular medium and of the endo/sarcoplasmic reticulum which allow the release of the ion from the intracellular stores to the cytoplasm. Mitochondria that are located close enough to such channels could be transiently exposed to very high- Ca^{2+} concentrations, sufficient to activate their low-affinity Ca^{2+} uptake pathway. In these conditions mito-

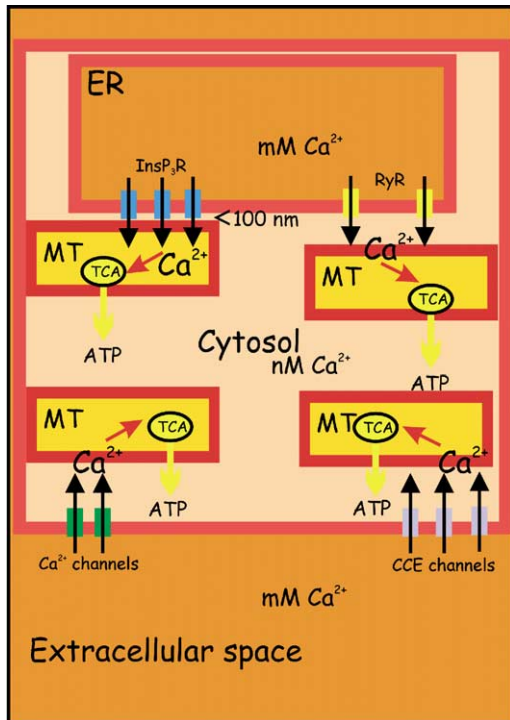


Fig. 1. Schematic model of the Ca^{2+} channels/mitochondria cross talk. Following cell stimulation Ca^{2+} is rapidly released from the ER via InsP_3 or Ry receptors and/or rapidly enters from the extracellular space via plasma membrane Ca^{2+} channels. In both cases a microdomain of high $[\text{Ca}^{2+}]$ is generated at the mouth of the channels which allows rapid Ca^{2+} uptake in the neighbouring mitochondria via the low-affinity mitochondrial uniporter, causing a rise in the $[\text{Ca}^{2+}]$ of the matrix. The rise in matrix $[\text{Ca}^{2+}]$ allows the activation of mitochondrial metabolism. ER: endoplasmic reticulum; MT: mitochondrion; CCE: capacitative calcium entry; TCA: tricarboxylic acid cycle.

chondria accumulate Ca^{2+} ions very rapidly and efficiently (Fig. 1). This aspect and its physiological meaning will be treated in details in the next paragraph.

3. Mitochondrial Ca^{2+} transport in living cells and its role in different cell types

The original observation that mitochondrial Ca^{2+} accumulation in living cells depended on the existence of microdomains of high $[\text{Ca}^{2+}]$ traced back to 10 years ago. Direct measurements of mitochondrial $[\text{Ca}^{2+}]$ with the selective recombinant probe aequorin [8] showed that, in HeLa cells, application of physiological stimuli coupled to the generation of InsP_3 , the physiological agonist which releases Ca^{2+} from the endoplasmic reticulum, caused a large, rapid rise in $[\text{Ca}^{2+}]$ in the mitochondrial matrix, $[\text{Ca}^{2+}]_m$ [11]. The evidence that $[\text{Ca}^{2+}]$ transients measured with aequorin were indeed $[\text{Ca}^{2+}]$ changes in the mitochondrial matrix was given by the fact that they were completely abolished in the presence of carbonylcyanide *p*-(trifluoro-methoxy)phenylhydrazone (FCCP), a

protonophore which collapses the proton gradient [8], and thus the driving force for Ca^{2+} accumulation in the matrix or following injection of the uniporter inhibitor RR [12].

Through the work of various laboratories and using different probes (recombinant aequorin or mitochondrially accumulated fluorescent dyes) this observation has been extended to a variety of cell types differing radically in the Ca^{2+} signalling pathway and in their embryological origins. There is a general consensus now that virtually in all cell types mitochondrial $[\text{Ca}^{2+}]$ rises are induced by cell stimulations that induce cytosolic $[\text{Ca}^{2+}]$ transients. Large Ca^{2+} rises were observed in primary cultures of skeletal myotubes upon opening the ryanodine receptors [13], in hepatocytes [14], in endothelial cells [15] or in chromaffin cells [16] upon opening the ER and the plasma membrane Ca^{2+} channels. It was also observed that in cardiomyocytes and astrocytes local Ca^{2+} release from intracellular stores induces highly localised transient mitochondrial depolarisations and mitochondrial Ca^{2+} uptake [17,18].

Now it is also well accepted that microheterogeneity exists in cellular Ca^{2+} concentration and that localised hot spots of high- Ca^{2+} concentration could be sensed by neighbouring mitochondria. This hypothesis has been initially suggested by experiments with digitonin-permeabilised cells [11]. In these experiments, HeLa cells, expressing recombinant mitochondrially targeted aequorin, were permeabilised with digitonin in a way that the functional interaction between ER and mitochondria were kept intact, and then perfused with InsP_3 or exposed to buffered $[\text{Ca}^{2+}]$ medium. The mitochondrial Ca^{2+} accumulation was rapid in the presence of InsP_3 and similar only when the cells were perfused with buffered $[\text{Ca}^{2+}]$ of 5–10 μM , i.e. 10- to 20-fold higher than that measured in the cytoplasm of stimulated cells. These experiments suggested that a close apposition of mitochondria to ER Ca^{2+} release channels (or to plasma membrane Ca^{2+} channels) was a necessary prerequisite for mitochondrial Ca^{2+} uptake. Two types of evidence later confirmed that this was the case. The first one was based on morphological data that gave the direct demonstration of the close apposition of the two compartments by high-resolution microscopy techniques. By combining the expression of ER targeted green fluorescent protein (GFP) and mitochondrially targeted blue fluorescent protein (BFP), a spectrally shifted mutant of the GFP, and using digital imaging microscopy it was demonstrated that, in living cells, ER and mitochondria were in close contact over about 5% of their surface [19] and that those contacts were estimated to be $< 100\text{ nm}$, at the limit of resolution of the imaging experiments. The second evidence was based on experiments in which the Ca^{2+} concentration at the mitochondrial surface was measured using an aequorin probe located between the outer and the inner mitochondrial membrane. This probe revealed that, after cell stimulation with an agonist coupled to InsP_3 production, larger $[\text{Ca}^{2+}]$ rises were evoked in the mitochondrial inter-membrane space in respect to those measured in the bulk cytosol [19].

The obvious functional significance of the mitochondria and ER (or other organelles) association and of the transient Ca^{2+} accumulation in the matrix is the activation of mitochondrial metabolism. From the biochemical studies on mitochondrial function dating over two decades ago [5,6], in fact, it was well known that two enzymes of the Krebs cycle (isocitrate and oxoglutarate dehydrogenase) and pyruvate dehydrogenase are regulated by matrix $[\text{Ca}^{2+}]$. More recent experimental evidence demonstrated the direct relationship between mitochondrial Ca^{2+} concentration rises and increases in NADH levels [12,14,20] and ATP production [21].

The use of recombinant aequorin has also permitted to analyse in detail the activation of the three matrix enzymes by Ca^{2+} , and to correlate it with the matrix free $[\text{Ca}^{2+}]$. $[\text{Ca}^{2+}]_m$ and NADH production were measured in living HeLa cells upon agonist-stimulation and, indeed, a marked increase in NADH production was observed in parallel with transient $[\text{Ca}^{2+}]_m$ rises [12]. An interesting observation was reported in hepatocytes where it was documented that InsP_3 -dependent $[\text{Ca}^{2+}]_c$ oscillations induced mitochondrial $[\text{Ca}^{2+}]$ oscillations as well, which, if their frequency was sufficiently high, were translated in a sustained increase in NADH production [14]. In this respect, the mitochondria act as decoder of cytosolic $[\text{Ca}^{2+}]$ oscillations. This mechanism appeared to be finely tuned on the frequency in the $[\text{Ca}^{2+}]$ oscillations: low-frequency oscillations corresponds to NADH oscillations, high-frequency Ca^{2+} oscillations corresponds to sustained increase, suggesting that the enzymes remained activated also during the inter-spike periods. Similar phenomena were observed in pancreatic β -cells and adrenal glomerulosa cells where $[\text{Ca}^{2+}]_c$ oscillations were associated with oscillatory changes in NADH [20]. The correlation between the changes of mitochondrial $[\text{Ca}^{2+}]$ and ATP production has been directly demonstrated recently using recombinant targeted luciferase [21]. In both HeLa cells and primary cultures of skeletal myotubes, stimulation with agonists evoking cytosolic and mitochondrial Ca^{2+} signals caused increases in mitochondrial and cytosolic [ATP] that depended on the amplitude of Ca^{2+} rises in the mitochondrial matrix and the availability of mitochondrial substrates. Interestingly, the authors suggested that Ca^{2+} elevation induced a long-lasting priming that persisted up to 1 h after cell stimulation and represented a sort of cell memory, since the addition of oxidative substrates caused a major increase in mitochondrial [ATP].

The ubiquitous and probably most important consequence of mitochondrial Ca^{2+} accumulation is the activation of key matrix enzymes. However, it is becoming evident that this is only one of the effects on the cellular processes controlled by mitochondrial Ca^{2+} (Fig. 2). In particular, the clearing of local $[\text{Ca}^{2+}]_c$ in the Ca^{2+} release channels proximity plays a role in the modulation of the Ca^{2+} channels activity. The first example of this effect was reported in *Xenopus* oocytes [22] where, by buffering microdomains of $[\text{Ca}^{2+}]_c$, mitochondria regulated the open probability of InsP_3 channels, relieving

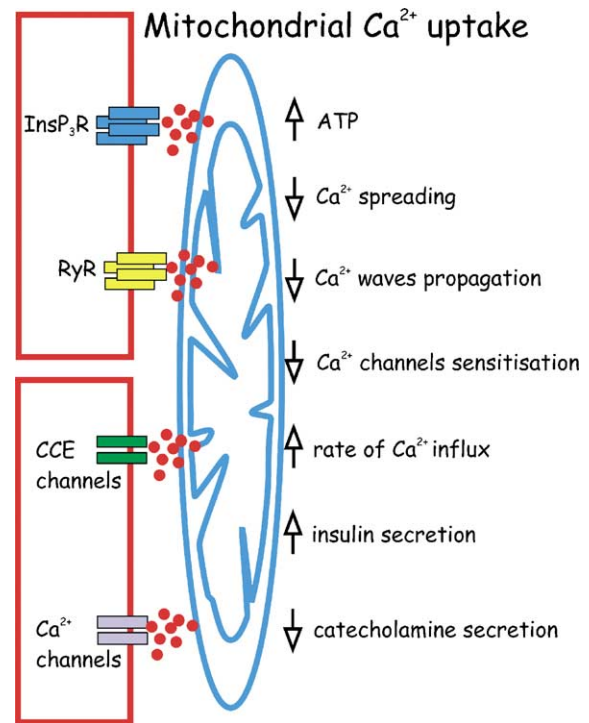


Fig. 2. Schematic representation of the interplay between mitochondrial Ca^{2+} uptake and shaping of cytosolic Ca^{2+} signalling. The local removal of Ca^{2+} by buffering mitochondria modulates the open probability of the Ca^{2+} channels, which in turn regulates the rate, and the spreading of Ca^{2+} signals.

the $[\text{Ca}^{2+}]$ inhibitory effect [23]. As a consequence, the rate of Ca^{2+} efflux from ER was modulated and, in turn, the shape of cytoplasmic Ca^{2+} waves. A similar role for mitochondria has been described also in mammalian cells where mitochondria suppressed $[\text{Ca}^{2+}]$ positive (or negative) effects on the InsP_3 or ryanodine channels [24,25].

Evidences have also been reported for a role of mitochondria in modulating the activity of plasma membrane channels. Lewis and co-workers [26] reported that in leukemic Jurkat T cells mitochondria can sense $[\text{Ca}^{2+}]_c$ gradients near Ca^{2+} release-activated Ca^{2+} (CRAC) channels and they can reduce the high- Ca^{2+} microdomains generated in the proximity of the mouth of the channels and thus the Ca^{2+} feedback inhibition. By dissipating the local $[\text{Ca}^{2+}]$ microdomains mitochondria can buffer the Ca^{2+} that enters T cells via store-operated Ca^{2+} channels: they sequester Ca^{2+} during periods of rapid Ca^{2+} entry and release it slowly after Ca^{2+} entry has ceased. The idea that by slowly releasing stored Ca^{2+} mitochondria can prolong the period of $[\text{Ca}^{2+}]_c$ elevation in response to a transient episode of Ca^{2+} influx was already documented in the literature [27–29] and it was suggested that these actions may have important effects on Ca^{2+} -dependent processes such as exocytosis and synaptic transmission. In their paper, Lewis and co-workers have also shown that mitochondria play an active role in modulating the rate of Ca^{2+} influx: cell treatment

with inhibitors of mitochondrial Ca^{2+} transport prevented the ability of T cells to maintain a high rate of capacitative Ca^{2+} entry.

The local buffering of $[\text{Ca}^{2+}]_c$ by mitochondria has been shown to have an important role in chromaffin cells in the modulation of catecholamine secretion [16]. By using aequorin mutants with different affinities, Alvarez and co-workers have shown that inhibition of Ca^{2+} sequestration by mitochondria causes a large increase in the secretion of catecholamine, suggesting that mitochondrial buffering of Ca^{2+} represents a way to control the recruitment or the fusion of catecholamine-containing secretory vesicles. They also have shown that, after cell stimulation, mitochondria can accumulate large amounts of Ca^{2+} (reaching concentration in the millimolar range) without impairing mitochondrial function, and that, in intact cells, two different mitochondrial populations can be identified which differ in their localisation with respect to Ca^{2+} hotspot sources. Those which are close to regions of high-cytosolic concentrations show large increases in $[\text{Ca}^{2+}]_m$ and the others essentially do not take up Ca^{2+} .

The control of the ion diffusion throughout the cell has been revealed another important aspect of mitochondrial Ca^{2+} signalling. This aspect seems to be a general mechanism to control the shaping of cytosolic Ca^{2+} signal: it has been in fact described in various cell types. Petersen and co-workers [30] demonstrated that in pancreatic acinar cells mitochondria strategically located beneath the granular region prevent the spreading of a Ca^{2+} wave from the secretory pole towards the basolateral region by accumulating Ca^{2+} . Duchen and co-workers [31] reported a similar role for mitochondria in rat cortical astrocytes. They suggested that mitochondria exert a negative feedback on the propagation of intracellular Ca^{2+} waves. By digital fluorescence imaging techniques they have shown that preventing mitochondria Ca^{2+} uptake by dissipating the mitochondrial membrane potential slowed the rate of decay of $[\text{Ca}^{2+}]_c$ transients and significantly increased the rate of propagation of Ca^{2+} waves.

4. Pathophysiological alterations of mitochondrial Ca^{2+} signalling

Mitochondria play such a significant role in shaping cellular Ca^{2+} signals in all cell types that any disturbance either in the organelle distribution and morphology or in the driving force for Ca^{2+} accumulation may result in impaired cell function. Indeed, in a number of neurodegenerative diseases it has been described that mitochondrial disorders may be implicated in the pathogenesis [32,33]. However, it is still difficult to establish whether the alterations in mitochondrial function are the cause or the effect of the disease. I discuss here only a few examples where an alteration of mitochondrial Ca^{2+} handling has been shown to generate the cellular phenotypes.

Mitochondrial diseases (also referred as mitochondrial encephalomyopathies) include a number of genetic disorders of mitochondrial genome (mtDNA) [34]. However, if many of molecular defects have been identified, the pathways that lead to cellular and clinical dysfunctions are largely unknown. Recently, experiments in transmitochondrial cell lines (“cybrids”), in which ρ^0 cells (where mtDNA has been destroyed) are fused with cells carrying the tRNA^{lys} mutation of MERRF (myoclonic epilepsy with ragged red fibres) or the ATPase6 mutation of NARP (neuropathy, ataxia and retinitis pigmentosa) showed that mitochondrial Ca^{2+} responses to agonists was drastically reduced in MERRF cybrids but not in NARP cells. The cytoplasmic response was normal in both the cases. These results were partially predictable since tRNA mutation affects all the mitochondrially encoded subunits of respiratory complexes thus impairing the generation of the proton gradient across the mitochondrial membrane (and thus the driving force for Ca^{2+} accumulation), while ATPase mutation do not. Interestingly, the impairment of Ca^{2+} responses caused a decrease in ATP production following cell stimulation, which could be restored by applying an inhibitor of the mitochondrial Ca^{2+} efflux pathways. This indicates that a defect in mitochondrial Ca^{2+} homeostasis is a relevant event in the cellular pathogenesis of the MERRF disorder [35].

The role of mitochondrial Ca^{2+} in modulating ATP levels has been described to have a fundamental role in insulin secreting cells, the pancreatic β -cells [36,37], since in turn ATP level controls the gating of ATP sensitive K^+ channels and thus the initiation of the secretion process. Wollheim and co-workers showed that a reduction in mitochondrial Ca^{2+} accumulation is responsible for a reduction in insulin secretion suggesting that modulation of mitochondrial Ca^{2+} uptake may be involved in the desensitisation of insulin secretion in response to repeated exposures to metabolic substrates [38]. They also suggested that a mitochondrial factor, distinct from ATP, and later identified as glutamate [39] participated in the triggering of insulin exocytosis. The central role of mitochondria in the transduction pathway is further underlined by the observation that diabetes mellitus is very common in patients affected by mitochondrial encephalomyopathies and by the finding that maternally inherited form of diabetes is associated to mtDNA mutations [40,41].

Very recently it has been shown that early mitochondrial Ca^{2+} defects are directly involved in the pathogenesis of the Huntington’s disease (HD) [42]. It was demonstrated that mutant huntingtin, containing an expanded polyglutamine tract, reduced mitochondrial membrane potential in normal lymphoblast mitochondria, thus impairing the capacity of mitochondria to take up Ca^{2+} . This finding partially reproduced the defects seen in HD patients and in HD mutant transgenic mice, where the authors described that mitochondria depolarised much more readily than control mitochondria. Altered mitochondrial distribution was observed in neuronal process of motoneuronal cells expressing mutated androgen receptor, a model for spinal and

bulbar muscular atrophy [43]. Since neuronal degeneration is linked to the same type of mutation of HD in unrelated genes (abnormal expansion of a polyglutamine tract normally present in the wild-type proteins) it can be envisaged that abnormal polyglutamine tract per se is responsible for mitochondrial alterations and that similar mitochondrial defects (both in the function and in the distribution) may occur in other polyglutamine disorders.

Apart from a series of specific pathologies, mitochondria have been proposed recently to play a central role in programmed cell death [44]. In most pathways leading to apoptosis the release of proapoptotic factors located in the inter-membrane space of the mitochondria or in their matrix (e.g. cytochrome c, AIF, caspase-2 and -9) represents a key step in the progression of cell death [45,46]. It was proposed that mitochondrial Ca^{2+} overload together with oxidative stress is a potent stimulus for the PTP opening which in turn seems to represent the major route for the release of the caspase co-factors [47].

It is not yet clear how increases in $[\text{Ca}^{2+}]$, which regulate a diverse range of cellular processes, can be a signal for both physiological events as well as cell death [48]. Interestingly on this topic, Hajnoczky and co-workers recently showed how apoptotic stimuli induce a switch in mitochondrial calcium signalling at the beginning of the apoptotic process. They proposed that in cells exposed to proapoptotic stimuli the Ca^{2+} sensitivity of PTP increases and that cell stimulation with an InsP_3 -generating agonist induces $[\text{Ca}^{2+}]_m$ transients which in turn cause the PTP opening. In their model prolonged exposure to proapoptotic stimuli or large Ca^{2+} overload are not necessary to induce cell death, but rather PTP opening is the consequence of a coincident detection of short-living signals: it is dependent on a privileged Ca^{2+} signal transmission between InsP_3 receptor and mitochondria [49], in addition to a yet unidentified direct effect of the proapoptotic lipid on mitochondria. Moreover, mitochondrial derangements have been shown to be preliminary signs of ceramide-induced apoptosis that is prevented by all the experimental conditions that reduced the amount of Ca^{2+} releasable from the intracellular stores [50].

5. Conclusions

Thanks to the development of imaging techniques which allow single cell analysis and of selective probes for measuring $[\text{Ca}^{2+}]$ in the mitochondrial matrix it has clearly emerged that mitochondria play a major role both in the physiology and in the pathophysiology of cells. The close relationship between mitochondria and the cellular Ca^{2+} pools allows them to efficiently accumulate Ca^{2+} ions in their matrix despite the low affinity of their Ca^{2+} uptake pathway. The direct consequence of mitochondrial Ca^{2+} uptake is the activation of mitochondrial metabolism. However, it became clear that this is only one of the aspects controlled by mitochondrial Ca^{2+} signalling.

Mitochondria strategically located contribute to the dissipation of local microdomains of high $[\text{Ca}^{2+}]$ close to the ER Ca^{2+} release channels and the plasma membrane Ca^{2+} entry channels thus modulating the activity of the channels and counteracting the spreading of cytosolic Ca^{2+} signal. In this respect mitochondria may be considered as spatial buffer and it can be speculated that their distribution could change in response to specific cell demands. It is obvious that disturbance of mitochondrial Ca^{2+} homeostasis could evoke different pathological phenotypes that are not only directly linked to the impairment of energetic metabolism.

Acknowledgements

I wish to thank Rosario Rizzuto for helpful discussion and critical reading of the manuscript. The experimental work in the author's laboratory was supported from Telethon (Grant no. GP0193Y01), the Italian University and Health Ministries and the National Research Council of Italy (CNR, Agency 2000).

References

- [1] D.E. Clapham, Calcium signalling, *Cell* 80 (1995) 259–268.
- [2] M.J. Berridge, P. Lipp, M.D. Bootman, The versatility and universality of calcium signalling, *Nature* 1 (2000) 11–21.
- [3] E. Carafoli, L. Santella, D. Branca, M. Brini, Generation, control, and processing of cellular calcium signals, *Crit. Rev. Biochem. Mol. Biol.* 36 (2001) 107–260.
- [4] H. Streb, R.F. Irvine, M.J. Berridge, I. Schulz, Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol 1,4,5-trisphosphate, *Nature* 306 (1983) 67–69.
- [5] J.G. MacCormack, A.P. Halestrap, R.M. Denton, Role of calcium ions in regulation of mammalian intramitochondrial metabolism, *Physiol. Rev.* 70 (1990) 391–425.
- [6] R.G. Hansford, Physiological role of mitochondrial Ca^{2+} transport, *J. Bioenerg. Biomembr.* 26 (1994) 495–508.
- [7] P. Bernardi, Mitochondrial transport of cations: channels, exchanger and permeability transition, *Physiol. Rev.* 79 (1999) 1127–1155.
- [8] R. Rizzuto, A.W.M. Simpson, M. Brini, T. Pozzan, Rapid changes of mitochondrial Ca^{2+} revealed by specifically targeted recombinant aequorin, *Nature* 358 (1992) 325–328.
- [9] A. Minta, T. Kao, R.Y. Tsien, Fluorescent indicators for cytosolic calcium based on rhodamine and fluorescein chromophores, *J. Biol. Chem.* 264 (1989) 8171–8178.
- [10] H. Miyata, H.S. Silverman, S.J. Sollot, E.G. Lakatta, M.D. Stern, R.G. Hansford, Measurement of mitochondrial free Ca^{2+} concentration in living single rat cardiac myocytes, *Am. J. Physiol.* 261 (1991) 1123–1134.
- [11] R. Rizzuto, M. Brini, M. Murgia, T. Pozzan, Microdomains of high Ca^{2+} close to inositol-triphosphate sensitive channels are sensed by neighbouring mitochondria, *Science* 262 (1993) 744–747.
- [12] R. Rizzuto, C. Bastianutto, M. Brini, M. Murgia, T. Pozzan, Mitochondrial Ca^{2+} homeostasis in intact cells, *J. Cell Biol.* 126 (1994) 1183–1194.
- [13] M. Brini, F. De Giorgi, M. Murgia, R. Marsault, M.L. Massimino, M. Cantini, R. Rizzuto, T. Pozzan, Subcellular analysis of Ca^{2+} homeostasis in primary cultures of skeletal muscle myotubes, *Mol. Biol. Cell* 8 (1997) 129–143.

- [14] G. Hajnoczky, L.D. Robb-Gaspers, M.B. Seitz, A.P. Thomas, Decoding of cytosolic Ca^{2+} oscillations in the mitochondria, *Cell* 82 (1995) 415–424.
- [15] A.M. Lawrie, R. Rizzuto, T. Pozzan, A.W.M. Simpson, A role for calcium influx in the regulation of mitochondrial calcium in endothelial cells, *J. Biol. Chem.* 271 (1996) 10753–10759.
- [16] M. Montero, M.T. Alonso, E. Carmicero, I. Cuchillo, A.G. Garcia, J. Garcia-Sancho, J. Alvarez, Millimolar $[\text{Ca}^{2+}]$ transients in mitochondria close to couplings of Ca^{2+} entry and Ca^{2+} release, *Nat. Cell. Biol.* 2 (2000) 57–60.
- [17] M.R. Duchen, A. Leyssens, M. Crompton, Transient mitochondrial depolarisations in response to focal SR calcium release in single rat cardiomyocytes, *J. Cell. Biol.* 142 (1998) 975–988.
- [18] D. Jacobson, M.R. Duchen, Fluorescence imaging of the mitochondrial permeability transition in rat cortical astrocytes in culture, *J. Physiol.* 506 (1998) 75.
- [19] R. Rizzuto, P. Pinton, W. Carrington, F.S. Fay, K.E. Fogarty, L.M. Lifshitz, R.A. Tuft, T. Pozzan, Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses, *Science* 280 (1998) 1763–1766.
- [20] W.F. Pralong, A. Spat, C.B. Wollheim, Dynamic pacing of cell metabolism by intracellular Ca^{2+} transients, *J. Biol. Chem.* 269 (1994) 27310–27314.
- [21] L.S. Jouaville, P. Pinton, C. Bastianutto, G.A. Rutter, R. Rizzuto, Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming, *Proc. Natl. Acad. Sci. U.S.A.* 96 (1999) 13807–13812.
- [22] L.S. Jouaville, F. Ichas, E.L. Holmuhamedov, P. Camacho, J.D. Lechleiter, Synchronization of calcium waves by mitochondrial substrates in *Xenopus levis* oocytes, *Nature* 377 (1995) 438–441.
- [23] I. Bezprozvanny, J. Watras, B.E. Ehrlich, Bell-shaped calcium response curves of $\text{Ins}(1,4,5)\text{P}_3$ and calcium gated channels from endoplasmic reticulum of cerebellum, *Nature* 351 (1991) 751–754.
- [24] B. Landolfi, S. Curci, L. Debellis, T. Pozzan, A. Hofer, Ca^{2+} homeostasis in the agonist-sensitive internal store: functional interactions between mitochondria and the ER measured in situ in intact cells, *J. Cell. Biol.* 142 (1998) 1235–1243.
- [25] G. Hajnoczky, R. Hager, A.P. Thomas, Mitochondria suppress local feedback activation of inositol 145-trisphosphate receptors by Ca^{2+} , *J. Biol. Chem.* 274 (1999) 14157–14162.
- [26] M. Hoth, C.M. Fanger, R.S. Lewis, Mitochondrial regulation of store operated calcium signalling in T lymphocytes, *J. Cell. Biol.* 137 (1997) 633–648.
- [27] S.A. Thayer, R.J. Miller, Regulation of the intracellular free calcium concentration in single rat dorsal root ganglion neurones in vitro, *J. Physiol.* 425 (1990) 85–115.
- [28] D.D. Friel, R.W. Tsien, An FCCP sensitive Ca^{2+} store in bullfrog sympathetic neurons and its participation in stimulus-evoked changes in $[\text{Ca}^{2+}]_c$, *J. Neurosci.* 14 (1994) 4007–4024.
- [29] D.F. Babcock, J. Herrington, P.C. Goodwin, Y.B. Park, B. Hille, Mitochondrial participation in the intracellular Ca^{2+} network, *J. Cell. Biol.* 136 (1997) 833–844.
- [30] H. Tinel, J.M. Cancela, H. Mogami, J.V. Geransimenko, O.V. Geransimenko, A.V. Tepikin, O.H. Petersen, Active mitochondria surrounding the pancreatic acinar granule region prevent spreading of inositol trisphosphate evoked local cytosolic Ca^{2+} signals, *EMBO J.* 18 (1999) 4999–5008.
- [31] E. Boiter, R. Rea, M.R. Duchen, Mitochondria exert a negative feedback on the propagation of intracellular Ca^{2+} waves in rat cortical astrocytes, *J. Cell. Biol.* 145 (1999) 795–808.
- [32] M.F. Beal, Mitochondrial dysfunction in neurodegenerative diseases, *Biochim. Biophys. Acta* 1366 (1998) 211–223.
- [33] C. Krieger, M.R. Duchen, Mitochondria, Ca^{2+} and neurodegenerative disease, *Eur. J. Pharm.* 447 (2002) 177–188.
- [34] A.H.V. Schapira, The “new” mitochondrial disorders, *J. Neurol. Neurosurg. Psychiatry* 72 (2002) 144–149.
- [35] M. Brini, P. Pinton, M.P. King, M. Davidson, E.A. Schon, R. Rizzuto, A calcium signalling defect in the pathogenesis of a mtDNA-inherited oxidative phosphorylation deficiency, *Nature Med.* 5 (1999) 951–954.
- [36] E.D. Kennedy, C.B. Wollheim, Role of mitochondrial calcium in metabolism-secretion coupling in nutrient stimulated insulin release, *Diabetes Metabol.* 24 (1998) 15–24.
- [37] G.A. Rutter, R. Rizzuto, Regulation of mitochondrial metabolism by ER Ca^{2+} release: an intimate connection, *Trends Biochem. Sci.* 25 (2000) 215–221.
- [38] P. Maechler, E.D. Kennedy, H. Wang, C.B. Wollheim, Desensitization of mitochondrial Ca^{2+} and insulin secretion responses in the beta cell, *J. Biol. Chem.* 273 (1998) 20770–20778.
- [39] P. Maechler, C.B. Wollheim, Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis, *Nature* 402 (1999) 685–689.
- [40] S.W. Ballinger, J.M. Shoffner, E.V. Hedaya, I. Trounce, M.A. Polak, D.A. Koontz, D.C. Wallace, Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion, *Nat. Genet.* 1 (1992) 11–15.
- [41] J.M. van den Ouweland, H.H. Lemkes, W. Ruitenbeek, L.A. Sandkuijl, M.F. de Vijlder, P.A. Struyvenberg, J.J. van de Kamp, J.A. Maassen, Mutation in mitochondrial tRNA^{leu} (UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness, *Nat. Genet.* 1 (1992) 368–371.
- [42] A.V. Panov, C.A. Gutekunst, B.R. Leavitt, M.R. Hayden, J.R. Burke, W.J. Strittmatter, J.T. Greenamyre, Early mitochondrial calcium defects in Huntington’s disease are direct effect of polyglutamines, *Nat. Neurosci.* 5 (2002) 711–712.
- [43] F. Piccioni, P. Pinton, S. Simeoni, P. Pozzi, U. Fascio, G. Vismara, L. Martin, R. Rizzuto, A. Poletti, Androgen receptor with elongated polyglutamine tract forms aggregates that alter axonal trafficking and mitochondrial distribution in motor neuronal processes, *FASEB J.* 16 (2002) 1418–1420.
- [44] M.R. Duchen, Contributions of mitochondria to animal physiology: from homeostatic sensor to calcium signalling and cell death, *J. Physiol.* 16 (1999) 1–17.
- [45] D. Green, G. Kroemer, The central executioners of apoptosis: caspases or mitochondria? *Trends Cell. Biol.* 8 (1998) 267–271.
- [46] S.L. Budd, L. Tennesi, T. Lishnak, S.A. Lipton, Mitochondrial and extramitochondrial apoptotic signaling pathways in cerebrocortical neurons, *Proc. Natl. Acad. Sci. U.S.A.* 97 (2000) 6161–6166.
- [47] P. Bernardi, L. Scorrano, R. Colonna, V. Petronilli, F. Di Lisa, Mitochondria and cell death. Mechanistic aspects and methodological issues, *Eur. J. Biochem.* 264 (1999) 687–701.
- [48] M.J. Berridge, M.D. Bootman, P. Lipp, Calcium—a life and death signal, *Nature* 395 (1998) 645–648.
- [49] G. Szalai, R. Krishnamurthy, G. Hajnoczky, Apoptosis driven by IP_3 -linked mitochondrial calcium signals, *EMBO J.* 18 (1999) 6349–6361.
- [50] P. Pinton, D. Ferrari, E. Rapizzi, F. Di Virgilio, T. Pozzan, R. Rizzuto, The Ca^{2+} concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the molecular mechanism of Bcl-2 action, *EMBO J.* 20 (2001) 2690–2701.