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Review

Ca^{2+} transfer from the ER to mitochondria: When, how and why

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ABSTRACT

The heterogenous subcellular distribution of a wide array of channels, pumps and exchangers allows extracellular stimuli to induce increases in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$) with highly defined spatial and temporal patterns, that in turn induce specific cellular responses (e.g. contraction, secretion, proliferation or cell death). In this extreme complexity, the role of mitochondria was considered marginal, till the direct measurement with targeted indicators allowed to appreciate that rapid and large increases of the $[Ca^{2+}]$ in the mitochondrial matrix ($[Ca^{2+}]_m$) invariably follow the cytosolic rises. Given the low affinity of the mitochondrial Ca^{2+} transporters, the close proximity to the endoplasmic reticulum (ER) Ca^{2+}-releasing channels was shown to be responsible for the prompt responsiveness of mitochondria. In this review, we will summarize the current knowledge of: i) the mitochondrial and ER Ca^{2+} channels mediating the ion transfer, ii) the structural and molecular foundations of the signaling contacts between the two organelles, iii) the functional consequences of the $[Ca^{2+}]_m$ increases, and iv) the effects of oncogene-mediated signals on mitochondrial Ca^{2+} homeostasis. Despite the rapid progress carried out in the latest years, a deeper molecular understanding is still needed to unlock the secrets of Ca^{2+} signaling machinery.

1. Calcium signaling and mitochondria: the general framework

In all eukaryotic cells, the cytosolic concentration of Ca^{2+} ($[Ca^{2+}]_c$) is tightly controlled by complex interactions among pumps, channels, exchangers and binding proteins, and relatively small and/or local changes in its concentration modulate a wide range of intracellular actions. $[Ca^{2+}]_c$ in resting condition is maintained around the value of 100 nM, significantly lower than extracellular $[Ca^{2+}]$ (1 mM). This condition is guaranteed by the low permeability of the plasma membrane to ions and by the activity of the Plasma Membrane Ca^{2+}-ATPase (PMCA, which pumps Ca^{2+} outside the cells) and of the Na^+/Ca^{2+} exchanger (NCX). This fine regulation of $[Ca^{2+}]$ allows this ion to act as one of the most important second messenger in signal transduction pathways [1,2].

The increase of intracellular $[Ca^{2+}]$ can be elicited through two fundamental mechanisms: i) the Ca^{2+} mobilization from intracellular stores, mainly the endoplasmic reticulum (ER) and Golgi apparatus, or ii) the entry from the extracellular milieu. The main route inducing Ca^{2+} release from intracellular stores involves the IP_3 Receptor (IP_3R), a transmembrane protein located on the ER and Golgi membrane, which exposes on the cytosolic face the IP_3 binding site, while it forms a Ca^{2+} channel in the transmembrane domain. When extracellular soluble agonists binds a G-coupled protein receptor, diverse isoforms of phospholipase C (PLC) are activated producing inositol-1,4,5-trisphosphate (IP_3) from the hydrolysis of phosphatidylinositol 4,5 bisphosphate (PIP_2). The binding of IP_3 to its receptor induces its opening and the release of Ca^{2+} from ER and Golgi. IP_3R is not the only protein involved in Ca^{2+} release: ryanodine Receptor (RyR), for example, is a transmembrane protein located on the ER membrane and it is activated by the alchaloid ryanodine and by Ca^{2+} itself, while Sphingolipid Ca^{2+} release-mediating protein of the ER (SCaMPER) is activated by sphingosine-1-phosphate [3]. The cADPr and NAADP could also be listed as activators (or putative activators) of Ca^{2+} release from the ER [4]. Intracellular store depletion consequent to the opening of the IP_3R triggers the activation of an inward rectifying Ca^{2+} current from the extracellular space named capacitative Ca^{2+} entry (CCE). The molecular determinants of CCE have been identified in the very last few years and include an ER Ca^{2+} sensing protein (STIM) and specialized Ca^{2+} channels in the plasma membrane (Orai, for a recent review [5]). The second mechanism inducing intracellular Ca^{2+} increases involves the opening of the plasma membrane Ca^{2+} channels, which are traditionally grouped into three classes: the Voltage Operated Ca^{2+} channels (VOCs) which open following a decrease of membrane potential [6], the Receptor Operated Ca^{2+} channels (ROCs), also called ligand gated channels, which open following the binding of an external ligand [7] and the Second Messenger Operated Channels (SMOCs) which open following the

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binding of a second messenger on the inner surface of the membrane [8]. Once activated its downstream targets, Ca^{2+} has to be rapidly removed from cytosol to restore the resting conditions. So, the Ca^{2+} signal is terminated by the combined activity of Ca^{2+} extrusion mechanisms, such as PMCA and NCX, and mechanisms refilling the intracellular stores, like sarco-endoplasmic reticulum Ca^{2+} ATPases (SERCAs). It has long been known that mitochondria can rapidly accumulate Ca^{2+} down the electrochemical gradient established by the translocation of protons across the inner mitochondrial membrane (IMM), which is expressed as a membrane potential difference ($\Delta\psi_m$) of −180 mV (negative inside) under physiological conditions [9]. However, the accurate measurements of [Ca^{2+}] in resting cells revealed values well below the affinity of the mitochondrial transporters. Thus, the role of mitochondria in Ca^{2+} homeostasis was considered marginal (i.e. limited to conditions of cellular Ca^{2+} overload), till the development of specific and reliable probes directly reported major swings of mitochondrial [Ca^{2+}] [10]. While enlivening the interest in mitochondrial Ca^{2+} homeostasis, these data raised an apparent contradiction between the prompt response of the organelle and the low affinity of the transporter. Based on a large body of experimental evidence, it is now generally accepted that the key to the rapid Ca^{2+} accumulation rests in the strategic location of a subset of mitochondria, close to the opening Ca^{2+} channel. While, based on cell morphology, such proximity is expected, and indeed often observed, in neuronal processes, a close proximity between ER–resident Ca^{2+} channels and mitochondria in non-excitable cells implies the assembly of a dedicated signaling unit at the organelle interphase. In this review, we will focus on these cell types, summarizing the essential molecular components of the Ca^{2+} signaling machinery of the ER and the mitochondria, the functional role of the Ca^{2+} cross-talk and some emerging regulatory mechanisms.

2. The mitochondrial side: a molecular enigma

The molecular machinery of mitochondrial Ca^{2+} transport is still largely obscure. Indeed, accumulation into the matrix, and consequent release, occur via the activity of transport mechanisms that were functionally characterized in the 70 s but were never molecularly identified despite the extensive efforts in this direction. We and other groups extensively worked on this topic and what emerged was that the outer mitochondrial membrane (OMM, although traditionally considered freely permeable) is a critical determinant of the mitochondrial Ca^{2+} accumulation [11,12]. Thus, the mitochondrial Ca^{2+} uptake machinery will be discussed, starting from the channels of the OMM to the yet unidentified components of the inner membrane; in parallel we will focus on the high-conductance channel named permeability transition pore (PTP), most likely spanning both mitochondrial membranes and causing organelle swelling and permeabilization, which plays a major role in pathological conditions.

2.1. The voltage-dependent anion channel (VDAC)

Ca^{2+} import across the OMM occurs through VDAC, the most abundant protein of the outer mitochondrial membrane. Yeast possesses only one channel-forming isoform (but has also another *VDAC* gene that correctly inserts into OMM showing no channel activity), while higher multicellular organisms and mammals have three distinct *VDAC* genes (*VDAC1*, *VDAC2* and *VDAC3*), with *VDAC1* representing the best characterized one. These three isoforms show a substantial sequence homology (from 65 to 75% in identity) and similar structure, with the only exception of VDAC2 that has a longer (11 aminoacids) N-terminal tail [13]. Yeasts lacking *VDAC* gene cannot grow on non-fermentable medium, thus highlighting the relevance of this channel in mitochondrial function: reintroduction of any of the mammalian *VDAC* genes in this yeast strain can promptly restore growth defects [14,15]. VDAC is traditionally considered as a large, high-conductance, weakly anion-selective channel, fully opened at low potential (<20–30 mV), but switching to cation selectivity and lower conductance at higher potentials (the so-called "closed" state). Moreover, when reconstituted into liposomes, each isoform induced a permeability in the liposomes with a similar molecular weight cutoff (between 3400 and 6800 Da based on permeability to polyethylene glycol). The physiological relevance of the voltage gating properties of VDAC is however still matter of debates, since it requires the existence of a potential across the OMM: although some have assumed such a potential is not possible, others have proposed several clues in support of this hypothesis (discussed in [16]). Moreover, a number of reports show that numerous cytosolic components can significantly modulate VDAC gating properties, including NADH [17], members of Bcl-2 protein family [18], metabolic enzymes [19], chaperones [20] and cytoskeletal elements [21]. Direct *in situ* measurement of OMM permeability revealed only small conductance at resting condition, thus suggesting a "default" closed state of the channel [22]. However, also in the closed state, small cations such as K^+, Na^+ and Ca^{2+} are believed to freely permeate the channel, with relevant variations in the conductance of the channel. A recent work by Tan and Colombini, describes the higher permeability of VDAC to Ca^{2+} in the closed states (with low permeability to anionic metabolites), rather than the opened state. So VDAC closure promotes Ca^{2+} flux into mitochondria, with consequent permeability transition and cell death, in according with previous observations that VDAC closure is a pro-apoptotic signal [23,24]. These notions have a direct impact on mitochondrial Ca^{2+} transport, as variations in OMM permeability to Ca^{2+} can represent a bottleneck for the efficient ion transfer from the high [Ca^{2+}] microdomain generated by the opening of the IP_3R to the intermembrane space. In support of this view, we demonstrated that the overexpression of VDAC enhances Ca^{2+} signal propagation into the mitochondria increasing the extent of mitochondrial Ca^{2+} uptake, acting at the ER–mitochondria contact sites [11]. Further evidence of the role of the VDAC were given by the demonstration of the physical link, that boosts Ca^{2+} accumulation in mitochondria, between VDAC and the IP_3R, through the molecular chaperone glucose-regulated protein 75 (grp75) [25].

2.2. The mitochondrial calcium uniporter (MCU)

Mitochondrial Ca^{2+} uptake plays a key role in the regulation of many cell functions, ranging from ATP production to cell death. However, the molecular mechanism underlying this phenomenon has not yet been completely explained, indeed, while the contribution of OMM Ca^{2+} channels (VDAC) has been well characterized, little is known about the so-called mitochondrial Ca^{2+} uniporter (MCU). MCU is an highly selective ion channel located in the mitochondrial inner membrane, with a dissociation constant ≤2 nM over monovalent cations, reaching saturation only at supraphysiological $[Ca^{2+}]_c$. Ca^{2+} crosses the inner mitochondrial membrane through the MCU thanks to the considerable driving force represented by the negative transmembrane potential. Also Sr^{2+} and Mn^{2+} are conducted by MCU and the relative ion conductance is: $Ca^{2+} \approx Sr^{2+} \geq Mn^{2+} \approx Ba^{2+}$. Studies performed on isolated mitochondria allowed the identification of some regulatory molecules acting on MCU, in particular the most effective inhibitors are the hexavalent cation Rutenium Red (RuR) and its related compound RuR360; MCU is also modulated by aliphatic polyamines, such as spermine and aminoglycosides, and by the adenine nucleotides, in the order of effectiveness ATP>ADP>AMP (whereas the nucleoside adenosine is ineffective) [26] as well as several plant-derived flavonoids [27]. RuR could represent a potentially important tool for the MCU identification, but it showed some major drawbacks: indeed, it binds a broad array of glycoproteins and it is completely cell-impermeant so that, even at high concentrations (50 mM), it is almost ineffective in reducing the mitochondrial Ca^{2+} transients elicited by cell stimulation. Another important regulator of

MCU is Ca^{2+} itself. As demonstrated by Moreau and its group [27], in fact, MCU has a biphasic dependence on cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$): $[Ca^{2+}]_c$ increase can both activate or inactivate mitochondrial Ca^{2+} uptake. MCU activation by Ca^{2+} is mediated by the Ca^{2+}-dependent Calmodulin activation and by the following activation of its effector, Calmodulin-dependent Protein KinaseII (CaM kinaseII), as demonstrated by the impairment of mitochondrial Ca^{2+} uptake induced by KN-62, an inhibitor of CaM kinaseII. On the other hand, $[Ca^{2+}]_c$ increase then inactivates the uptake pathway. These two processes follow an distinct kinetic: the uptake induction occurs with a time constant of 6 s, while the inactivation occurs with a time constant of 17 s. This mechanism allows the mitochondrial Ca^{2+} oscillation, but it prevents an excessive mitochondrial Ca^{2+} accumulation when intracellular Ca^{2+} elevation is prolonged [28]. Further studies performed to clarify the mechanisms regulating Ca^{2+} homeostasis, suggest a role of the kinase-mediated network in the regulation of Ca^{2+} uptake: in particular, the different isoforms of protein kinase C (PKC), when overexpressed in HeLa cells, showed different effects on global Ca^{2+} signaling (e.g. PKCα, possibly through the previously reported PKC-mediated phosphorylation of IP_3Rs, reduces ER Ca^{2+} release [29], while the other PKC isoforms act on mitochondrial homeostasis: PKCβ reduces mitochondrial Ca^{2+} transients, whereas PKCζ potentiates them) [30]. A recent paper by Graier et al. suggested that the uncoupling proteins 2 and 3 (UCP2 and UCP3) of the IMM are essential for mitochondrial Ca^{2+} uptake since isolated liver mitochondria from UCP2 KO mice show no RuR-sensitive Ca^{2+} uniporter acivity [31]. However, attempts from other groups to reproduce these data failed [32], thus the role of UCPs in mitochondrial Ca^{2+} uptake should be regarded with caution. Finally, circumventing the low affinity of the MCU, another mode of Ca^{2+} influx into mitochondria was described by Sparagna et al. [33], defined rapid mode of uptake or RaM. This route should allow mitochondria to uptake large amounts of Ca^{2+} in short pulses, at least 300 times faster than through the MCU. It should transport Ca^{2+} only for a brief period during the initial part of the pulse and then be inactivated by Ca^{2+} binding to an external binding site. These prerogatives thus imply a marginal role in the total uptake of Ca^{2+} in the matrix, but could possibly generate local $[Ca^{2+}]_m$ microdomains near the site of the transporter, that could represent hotspots for the regulation of Ca^{2+}-sensitive matrix processes. This work was not followed up, and thus the molecular identity, and even the existence, of RaM is even more elusive than MCU. Indeed, it is sensitive to the same regulatory mechanisms (e.g. it is also inhibited by RuR), so the possibility remains open that it is simply a different functional state of the MCU [34].

2.3. Calcium extrusion

The efflux pathways were extensively studied in isolated organelles, and their functional properties are fairly well characterized. The mitochondrial Na^+/Ca^{2+} exchanger (mNCX) is similar to that found in the plasma membrane; it allows Ca^{2+} efflux and it is inhibited by Sr^{2+}, Ba^{2+}, Mg^{2+} or Mn^{2+}, and by a variety of compounds of pharmacological interest such as diltiazem, verapamil and other blockers of the voltage-dependent calcium channels, and more specifically by CGP37157 [35]. As to the stechiometry of the exchange, Ca^{2+} was reported to be transported out of mitochondria against values greater than those predicted for passive leak, and thus a $Ca^{2+}/3Na^+$ was postulated [36]. The H^+/Ca^{2+} exchanger (mHCX) is prevalent in non-excitable cells, and it extrudes Ca^{2+} against a gradient that is much higher than what thermodynamic parameters permit for an electroneutral H^+/Ca^{2+} exchanger [37,38]. These efflux pathways can become saturated with high matrix Ca^{2+} load, such that sustained and rapid Ca^{2+} influx can still lead to mitochondrial Ca^{2+} overload. In recent years, an "old" ionic pathway has re-attracted great interest: the high-conductance channel referred to as the "permeability transition pore" (PTP) is traditionally considered to mediate the so-called mitochondrial permeability transition (MPT). MPT is a Ca^{2+}-dependent process and it classically induced by a Ca^{2+} overload in matrix $[Ca^{2+}]$ finally resulting in organelle swelling. This notion, and the functional characterization of the process, became of general interest to cell biologists, when it became apparent that this process is likely to play a major role in cell death. Indeed, loss of mitochondrial components and/or collapse of ionic gradient may lead, not only to bioenergetic catastrophe (and thus cell death by necrosis), but also, in a more controlled route, to cell death through apoptosis, elicited by the release of cytochrome *c* and of the other mitochondrial proteins responsible for receptor-independent activation of effectors caspases (the so-called intrinsic pathway of apoptosis) [39]. Despite strenuous effort, also PTP does not escape the fate of molecular uncertainty. The pore is likely to be located in the inner-outer contact sites of the mitochondrial membranes and it is believed to be a multi-protein complex, even if the exact characterization of its molecular components is still lacking. By the analysis of PTP activators and inhibitors, the adenine nucleotide transferase (ANT), the voltage-dependent anion channel (VDAC) and cyclophilin D (Cyp D) were proposed as the most likely candidates. ANT was suggested as a possible candidate according to the observation that Bongkrekate, an agent which holds ANT in the *m* conformation (facing the matrix) inhibits PTP opening, while Atractyloside, which holds ANT in the *c* conformation (facing the cytosol) activates PTP opening [40]. However, the judicious analysis of the available knock-out mice for all three VDAC and ANT isoforms has cast doubts on their role as obligatory components of PTP. Mitochondria from $VDAC^{-/-}$ cells exhibit Ca^{2+} and oxidative stress-dependent permeabilization indistinguishable from wild-type mitochondria [35], and the same has been observed in mitochondria lacking ANT [41]. Another important inhibitor of PTP opening is the immunosuppressant Cyclosporine A (CsA). The target of CsA is the peptidyl–prolyl cis–trans isomerase cyclophilin D (CypD) [42]. In presence of high Ca^{2+} concentrations, CypD binds to IMM and to ANT, thus inhibiting ATP/ADP binding and inducing PTP opening; CsA can bind CypD, thus inhibiting its translocation to IMM and the opening of PTP. These data strongly argue for a regulatory role of CypD within the PTP, in particular it seems to sensitize it to mitochondrial Ca^{2+} loading [43–46]. Numerous molecules can modulate the opening of the PTP. In conjunction with a Ca^{2+} rise in the mitochondrial matrix, also pH, adenine nucleotides, free radicals and mitochondrial membrane potential ($\Delta\Psi_m$) participate in controlling pore opening. Ca^{2+} entering the mitochondrial matrix enhances PTP opening because of the presence of a Ca^{2+}-binding site, competitively inhibited by other ions such as Mg^{2+}, Sr^{2+} and Mn^{2+}, and by Pi (through an unknown mechanism, that is believed to involve the Cyp D) [47]. Finally, proapototic stimuli, such as C2-ceramide, can increase PTP sensitivity to Ca^{2+} regulation [48]. Given the relevance of these regulatory mechanisms, we refer to more detailed reviews for an accurate coverage of these topics [49,50].

3. Calcium release from cellular store: structure and function of the IP_3R

Many extracellular stimuli, such as hormones, growth factors, neurotransmitters, neutrophins, odorants, and light, function generating IP_3 through the phospholipase C isoforms, activated in different manners: G-protein coupled receptors (acting via PLCβ), tyrosine-kinase coupled receptors (PLCγ), an increase in Ca^{2+} concentration (PLCδ) or activated by Ras (PLCε) [51,52]. The final effector is the inositol 1,4,5 trisphosphate IP_3-sensitive receptor, a member of a superfamily of ion channels with six transmembrane domains, residing on the ER membrane. The opening of the channel is under dual control, by IP_3 and by Ca^{2+} itself, as will be discussed in more detail later.

From the structural point of view, several domains are recognized in the protein sequence, with different functions. These include the

IP_3-binding domain (IP_3BD), i.e. the minimal sequence sufficient for IP_3 binding, located near the N-terminus of the protein (aa 226–578). Interestingly, this protein domain contains armadillo-repeat protein structures that are engaged in protein–protein interactions, and mediates intramolecular interactions with other IP_3R domains as well as the association with other regulatory proteins. N-terminally to the IP_3BD, i.e. within aa 1–222, a suppressor region is located that inhibits ligand binding and thus lowers the global receptor IP_3 affinity in the physiological range. In the C-terminal portion, hydrophobic residues form the C-terminal transmembrane/pore-forming domain [53,54], and, between them, an internal coupling domain assures the signal of IP_3 binding is transferred to the channel-forming region, hence triggering its opening [55]. Finally, in their coupling/suppressor domains, the IP_3Rs possess consensus sequences for phosphorylation by numerous kinases, including Protein Kinase A (cAMP-dependent) [56], Protein Kinase B (Akt/PKB) [57], Protein Kinase G (cGMP-dependent) [58], calmodulin-dependent protein kinase II (CaMKII) [59], protein kinase C (PKC) [60], and various protein tyrosine kinases (PTK) [61].

Three isoforms of IP_3R encoded by different genes have been identified with different agonist affinities and tissue distribution [62–64]. Given that the affinity of the IP_3-binding core to its ligand is similar for the three isoforms, the tuning of the whole receptor's affinity appears to be due to the isotype-specific properties of the N-terminal suppressor domain [65].

Ca^{2+} regulates channel activity in a biphasic manner, depending on Ca^{2+} concentration: at low [Ca^{2+}] the ion exerts an activatory role, while it has an opposite inhibitory effect at high [Ca^2, thus allowing a fine dynamic feedback regulation during Ca^{2+} release [66,67]. In addition, also the ER Ca^{2+} content retains the capability to regulate the channel opening: in permeabilized hepatocytes, an increase in $[Ca^{2+}]_{er}$ enhances the sensitivity of IP_3R for its ligand, promoting also spontaneous Ca^{2+} release, but the nature of this direct regulation and the protein involved are still a matter of debate [68,69]. In this context, the tight spatial relationship between ER and mitochondria, and the capacity of the latter to rapidly clear the high [Ca^{2+}] microdomain generated at the mouth of the IP_3R, makes mitochondria an active player in the control of IP_3R function. The first clear demonstration of this concept came from the fine work of Lechleiter et al., who demonstrated that energized mitochondria, by regulating the kinetics of ER Ca^{2+} release, finely tune the spatio-temporal patterning of Ca^{2+} waves in *Xenopus* oocytes [70]. Then, the observation that Ca^{2+} uptake by mitochondria controls the [Ca^{2+}] microdomain at the ER/mitochondrial contacts and thus the kinetics of IP_3R activation/inactivation was extended to a variety of mammalian cell lines, e.g. hepatocytes [71], astrocytes [72] and BHK-21 cells [73], thus highlighting its general relevance.

Whereas IP_3 and Ca^{2+} are essential for IP_3R channel activation, other physiological ligands, such as ATP, are not necessary but can finely modulate the Ca^{2+}-sensitivity of the channel [74]. As for Ca^{2+}, the modulation of IP_3R by ATP is biphasic: at micromolar concentrations, ATP exerts a stimulatory effect, while inhibiting channel opening in the millimolar range [75,76].

Upon IP_3 production, IP_3Rs have been shown to cluster at the ER membranes: the size and composition of these clusters depend on the isoform involved, while the global IP_3 binding affinity is shared among the different isoforms [63,77]. Spontaneous clustering of IP_3Rs (in particular of IP_3R-2, due to its higher IP_3 affinity) have been proposed to be the underlying mechanism responsible for Ca^{2+} puffs observed in the cytoplasm [55]. The merging of discrete and localized $[Ca^{2+}]_c$ increases, due to the opening of clustered IP_3Rs [78], are called "Ca^{2+} puffs" [79]. Recruitment of neighboring IP_3Rs and combination of Ca^{2+} puffs results in Ca^{2+} waves, ensuring that the Ca^{2+} signal propagates to the entire cell [80], or remains confined to specific subcellular regions [81]. The effects triggered by tightly controlling the diffusion of a $[Ca^{2+}]_c$ signal elicited by IP_3R opening is well illustrated by pancreatic acinar cells. In this polarized cell type, the Ca^{2+} wave originates in the apical pole and may spread through the entire cell reaching the nucleus, determining gene transcription or cell death. Alternatively, the $[Ca^{2+}]_c$ signal can remain localized near the source, i.e. the apical pole, activating short-term effects such as secretion of enzyme-containing granules [82–85]. The fate of the [Ca^{2+}] signal (remaining localized in the apical region, or reaching the basal region) depends on, at first, the effectiveness of the stimulus and the further action of different second messengers, and secondly, on the "firewall" effect given by mitochondria. Indeed, in this case mitochondria were shown to cluster between the apical and basal pole of the cell, thus forming a fixed "Ca^{2+} buffer" that must be overwhelmed by robust Ca^{2+} wave in order to reach the basolateral area. This occurs in case of supramaximal stimulations, but also when pathological challenges (e.g. alcohol or bile acids) synergistically act on the cells [86].

4. The molecular and cellular definition of the ER/mitochondria contacts

Close appositions between ER and mitochondria have been observed in electron micrographs (EM) of fixed samples in many different cell types while experiments performed by our group had eventually confirmed the physical and functional coupling of these two organelles in living cells, by labelling the two organelles with targeted spectral variants of GFP (mtBFP and erGFP) [87]. These experiments revealed the presence of overlapping regions of the two organelles (thus establishing an upper limit of 100 nm for their distance) and allowed to estimate the area of the contact sites as 5–20% of total mitochondrial surface. More recently, electron tomography techniques allowed to estimate an even smaller distance (10–25 nm) as well as the presence of trypsin-sensitive (hence proteinaceous) tethers between the two membranes [88].

Unfortunately, very few of the relevant scaffolding or signaling proteins of the ER/mitochondria contacts have been identified, despite the growing interest on the topic. Nevertheless, novel candidates are rapidly being isolated and it can be envisaged that the molecular characterization will rapidly proceed, thanks to the validation of biochemical approaches in the isolation of a subcellular fraction containing the putative ER/mitochondria contacts. Indeed one of the major apparent known technical pitfall of subcellular fractionation, i.e. the "contamination" of the mitochondrial fraction with ER vesicles, has been demonstrated to be due to the actual co-segregation of stably associated mitochondrial and ER membranes. This has led to a more accurate separation, through density gradient centrifugations, of pure mitochondria from the so-called "mitochondria-associated membrane" (MAM), which have been originally shown to be enriched in enzymes involved in lipid transfer between ER and mitochondrial membranes (e.g. the import of phosphatidylserine (PS) into mitochondria) [89–91]. More recently, the same subcellular fraction has been shown to contain as well Ca^{2+} signaling elements of both organelles [25,92], thus supporting the central role of ER(or SR)/mitochondria cross-talk in signal transduction.

The molecular scenario is gradually adding new information, and we will here cite a few interesting examples, mainly involving chaperones. The role of glucose-regulated protein 75 (grp75) within the mitochondrial matrix as a molecular chaperone assisting the refolding of newly imported proteins was well established [93]. It was then reported that a pool of grp75 is not imported into the matrix, but has an extra-mitochondrial distribution [94]. We identified, in a two hybrid screen, grp75 as a VDAC interactor, and demonstrated that it mediates the molecular interaction of VDAC with the IP_3R, allowing a positive regulation of mitochondrial Ca^{2+} uptake. This implies a sort of conformational coupling between the Ca^{2+} channels of the two organelles, and highlights the importance of macromolecular complexes located in the MAM for this functional interaction (Fig. 1) [25]. Another intriguing example is that of sigma-1, a novel ER chaperone

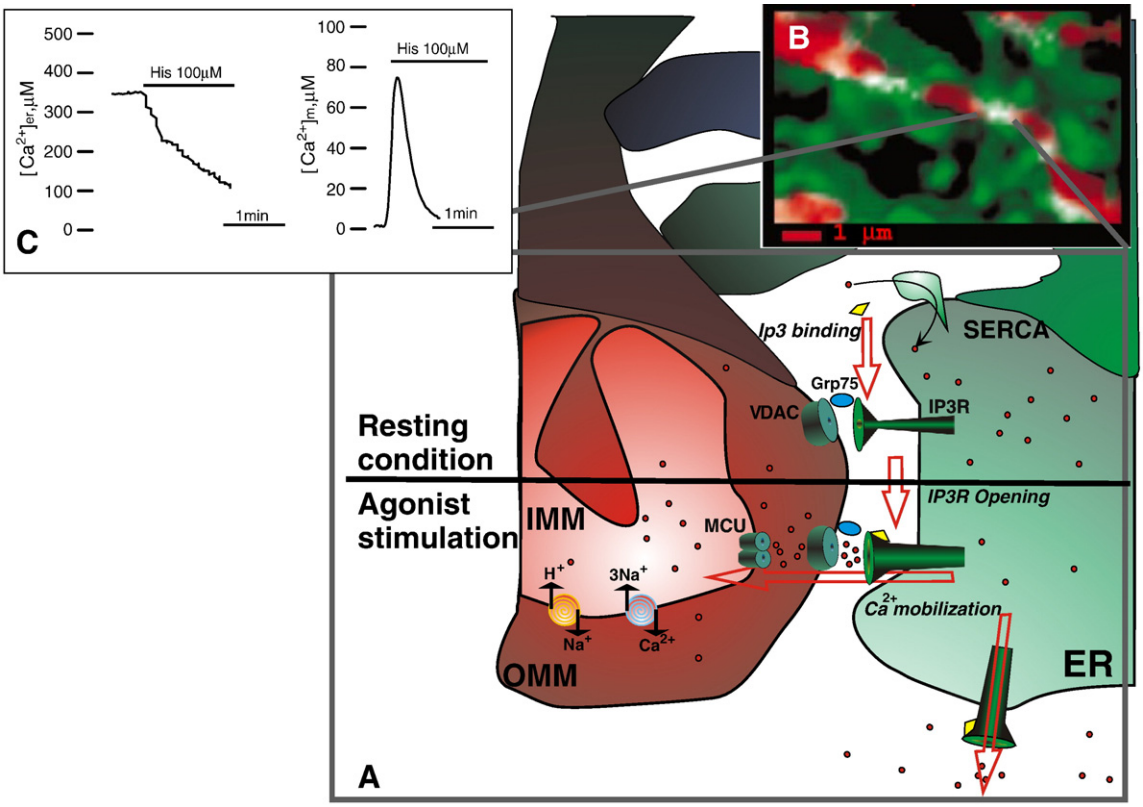

Fig. 1. (A) Schematical representation of mitochondria–ER contact sites and Ca^{2+} handling. Agonist stimulation induces IP_3 synthesis and consequently opening of IP_3R channel, which causes Ca^{2+} redistribution. SERCA, sarcoplasmic/endoplasmic calcium ATPase; VDAC, voltage-dependent anion channel; MCU, mitochondrial calcium uniporter; IP_3R, IP_3 receptor; (B) Combined 3D imaging of mitochondria and ER in a HeLa cell transiently expressing mtBFP(Y66H,Y145F) and erGFP(S65T) (5). (C) Representative traces of ER and mitochondrial calcium response during agonist stimulation measured using specifically targeted aequorins.

serendipitously identified in cellular distribution studies and shown to be involved in the Ca^{2+}-mediated stabilization of IP_3Rs [95]. Sigma-1 is normally localized in MAM, bound to another ER chaperone (BiP). When the luminal Ca^{2+} concentration of the ER drops, following the opening of IP_3Rs, sigma-1 dissociates from BiP and binds to IP_3R-3, thus preventing degradation by the proteasome. Thus, sigma-1 appears to be involved in maintaining, from the ER luminal side, the integrity of the ER/mitochondrial Ca^{2+} cross-talk in conditions (e.g. ER stress) that could impair signal transmission. In support of this notion, back in 2005, Simmen et al. reported the identification of a multifunctional sorting protein PACS-2, that integrates ER–mitochondria and apoptosis signaling; depletion of this protein causes mitochondrial fragmentation and uncoupling from ER, influencing Ca^{2+} homeostasis. Moreover in response of apoptotic stimuli, PACS-2 has been demonstrated to be capable of inducing Bid recruitment to mitochondria, event that leads to cytochrome *c* release and caspase 3 activation [96].

The shaping of the ER–mitochondrial network can be affected by binding proteins and physiological ligands; recently Hajnoczky et al. demonstrated that exposure to TGFβ affects Ca^{2+} transfer to the mitochondria through an impairment of the ER–mitochondrial coupling, thus supporting the notion of a highly dynamic regulation of inter-organelle communication [97].

This actively adapting interconnection stems also from the observation that these organelles are intrinsically highly dynamic structures continuously moving [98,99] and remodelling in their shape. As a consequence, the molecular determinants of this dynamism, such as for example, the family of "mitochondria-shaping proteins" (Drp1, mitofusins, Opa1 etc.) constitute potential modulators of ER/mitochondria cross-talk. Along this line, Scorrano et al. have recently pointed out the crucial role of the mitofusin (MFN 1 and 2), in particular the isoform 2 is thought to be important for ER–mitochondrial interactions engaging them in both homo and hetero-complexes [100]. They also showed that genetic ablation of MFN2 causes an increase in the distance between the two organelles with a consequent impairment of mitochondrial Ca^{2+} uptake, thus further supporting the high [Ca^{2+}] microdomains theory. Moreover the ER–mitochondrial apposition performed by MFN 2 predispose mitochondria to high Ca^{2+} microdomains and to the consequent overloading, leading eventually to apoptosis by excessive Ca^{2+} transfer.

5. Enhancing ATP production or killing the cell: the yin/yang of mitochondrial calcium

The main physiological role of Ca^{2+} uptake was assessed to be the control of metabolic activity of the mitochondria, in terms of ATP production rate. Indeed, important metabolic enzymes localized in the matrix, the pyruvate-, α-ketoglutarate- and isocitrate-dehydrogenases (collectively called the Ca^{2+}-sensitive mitochondrial dehydrogenases, CSMDHs) are activated by Ca^{2+}, with different mechanisms: the first through a Ca^{2+}-dependent dephosphorylation step, the others via direct binding to a regulatory site [101]. Those three enzymes represent rate-limiting steps of the Krebs cycle thus controlling the feeding of electrons into the respiratory chain and the generation of the proton gradient across the inner membrane, in turn necessary for Ca^{2+} uptake and ATP production. These events were directly visualized in intact, living cells using a molecularly engineered luciferase probe (a chimeric photoprotein including the mitochondrial targeting sequence derived from subunit VIII of cytochrome *c* oxidase). The probe revealed an increase in the [ATP] of the mitochondrial matrix following agonist stimulation and mitochondrial Ca^{2+} uptake [70]. Subsequent work revealed that this important example is only one of the mechanism controlling mitochondrial metabolism. Indeed, metabolite carriers of the inner membrane, such

as aralar1 and citrin, possess a Ca^{2+} binding site in the portion of the protein protruding in the intermembrane space, which is responsible for stimulation-dependent enhancement of substrate accumulation into the matrix [102]. This effect is lost if the Ca^{2+}-binding site is deleted from the carrier. Overall, these data indicate that a complex Ca^{2+}-sensing machinery, localized in different mitochondrial domains, underlies the coupling of aerobic metabolism to Ca^{2+}-mediated signals in the cytosol.

The interest in the process of mitochondrial Ca^{2+} homeostasis dramatically increased when it became apparent that also cell death is causally linked to organelle Ca^{2+} loading. On the one hand, it was clear that cellular Ca^{2+} overload, such as that caused by hyperstimulation of ionotropic glutamate receptors, leads to Ca^{2+} cycling across the mitochondrial membranes, collapse of the proton gradient and bioenergetic catastrophe, thus leading to cell death by necrosis. On the other hand, Ca^{2+} proved to sensitize cells to apoptotic challenges, acting on the mitochondrial checkpoint. This notion, subsequently confirmed by the study of other anti- and pro-apoptotic proteins, emerged from the analysis of the effect of Bcl-2 on Ca^{2+} signaling, as discussed later in this review. As discussed above Ca^{2+} binding to cyclophilin D positively regulates PTP opening [44] and in turn cell death [103]. Once opened, PTP allows the release in the cytosol of intermembrane-residing apoptotic factors, such as cytochrome *c*, AIF (apoptosis-inducing factor) and Smac/DIABLO, which can trigger apoptosis by both a caspase-dependent and a caspase-independent pathway [104]. Physiological $[Ca^{2+}]_m$ oscillations do not induce PTP opening, but become effective with the synergistic action of pro-apoptotic challenges (such as ceramide or staurosporin) [48,105].

As to differential effects of specific research effort, a deeper insight has been obtained for the IP_3R. The involvement of IP_3R in triggering apoptosis has been demonstrated in different cell types through IP_3R isoform-specific silencing in response to many apoptotic stimuli. In this intense research effort, type I and III isoforms were preferentially studied, while the role of IP_3R-2 in apoptosis, due to its low expression, limited to few human tissues, has not been clarified yet. In CHO cells, that expressed all three IP_3R isoforms, IP_3R-3 was shown to strongly co-localize with mitochondria and its silencing depressed agonist-dependent mitochondrial Ca^{2+} signals and apoptosis, triggered by different activators of the extrinsic or intrinsic pathway. Altogether, these data suggested that, at least in this cell type, this isoform could be primarily involved in transferring Ca^{2+} to mitochondria in apoptosis [106]. In other cell types, the experimental evidence calls for a preferential role of type I IP_3R. In Jurkat T lymphoma cells, ablation of IP_3R-1 protects cells from apoptosis induced by different apoptotic stimuli [107]. In agreement with this model, the co-culture of Jurkat T lymphoma cells with cells expressing high levels of Fas ligand, such as the W620 colon carcinoma cells, induces pro-apoptotic Ca^{2+} release from IP_3R to mitochondria in Jurkat cells [108]. Thus, it appears reasonable to conclude that, while IP_3R-mediated release of Ca^{2+} from ER appears a key sensitizing step in various apoptotic routes, the precise molecular definition of this process awaits the fine clarification of the macromolecular complex assembled at the interphase between the two organelles, since significant differences may occur in various cell types and/or physiological conditions.

6. Depressing ER/mitochondria Ca^{2+} cross-talk: an effective strategy for oncogenes?

6.1. Bcl2

Bcl-2 family members function as regulators of almost all known forms of programmed cell death. The first gene to be identified was *bcl-2*, encoding a protein of 26 kDa, rapidly characterized as an anti-apoptotic proto-oncogene [109]. In B-cell lymphomas, a chromosomal translocation causes the fusion of *bcl-2* gene with a transcriptional enhancer of immunoglobulin, with consequent accumulation of Bcl-2 protein, block of apoptosis and abnormal proliferation [110]. Then, a group of related proteins was identified, that share at least one of the four Bcl-2-homology (BH) domains. The presence/absence of one or more of these BH domains, distinguishes three different subfamilies of Bcl-2 related proteins: the anti-apoptotic subfamily (e.g. Bcl-2, Bcl-xL), which has all four of the BH domains, the multi-domain pro-apoptotic subfamily (e.g. Bax, Bak), lacking the BH4 domain only, and a pro-apoptotic subfamily (e.g. Bim, Bad) containing only the BH3 domain (BH3-only proteins) [111].

It is well beyond the scope of this review to cover the complex intervention in apoptosis of this protein family, and even of Bcl-2 itself. We will only review the growing body of evidence indicating a role of the oncoprotein in controlling the Ca^{2+} cross-talk between the ER and mitochondria. In Bcl-2 research focus was soon placed on intracellular organelles, when it was demonstrated that this oncoprotein is associated, within the cell, with the outer mitochondrial membrane, the ER and nuclear envelope, and also partly retained within the cytosol [112,113]. In the early 90s a few papers reported that Bcl-2 expression causes a greater resistance of mitochondria to Ca^{2+} induced respiratory injury, making cells competent to enter S-phase [114,115].

Approximately a decade ago, the proposal that Bcl-2 family members retain ion channel activity [116], triggered further work, by us and other labs, aimed at revealing alterations of Ca^{2+} homeostasis in the mitochondria and/or in the ER. Using a panel of organelle-targeted aequorin probes, we showed that overexpression of Bcl-2 reduces the state of filling of the agonist-sensitive Ca^{2+} stores (i.e. both the ER and the Golgi apparatus), by increasing Ca^{2+} leakage (Fig. 2A) [117]. This property was not shared by pro-apoptotic members of the protein family, and did not depend on the putative pore-forming domain, as demonstrated by experiments with wt Bax, and Bcl-2/Bax protein chimeras [118]. The mechanism was finally solved when it was demonstrated that Bcl-2 interacts with IP_3Rs and sensitizes them to low IP_3 concentrations [119]. As a consequence of the reduced filling, stimulus-dependent $[Ca^{2+}]$ increases were reduced both in the cytoplasm and in the mitochondria [117]. The same results were obtained using a different type of targeted indicator, the Ca^{2+}-sensitive GFP constructs. Using an ER-targeted 'CaMeleon', a lower steady-state $[Ca^{2+}]_{er}$ was measured in Bcl-2-overexpressing HeLa cells, as well as an increased leak rate upon thapsigargin blockage of SERCA pumps [120]. Then, using a similar probe, a CaMeleon with improved reaction kinetics and a K_d ideal for imaging Ca^{2+} in the ER, in MCF-7 breast cancer cells Bcl-2 was shown to lower $[Ca^{2+}]_{er}$ and to alter Ca^{2+} oscillations induced by ATP [121]. Finally, Bax/Bak double knock-out cells were shown to have a reduced $[Ca^{2+}]_{er}$, and hence to be protected from a variety of apoptotic challenges [122]. Bcl-2 silencing or SERCA overexpression restored both $[Ca^{2+}]_{er}$ and sensitivity to apoptotic challenges. These results are in keeping with previous work demonstrating that SERCA overexpression causes ER Ca^{2+}overload and increases spontaneous apoptosis [123]. However, in other experimental conditions and/or cell type, no effect of Bcl-2 on $[Ca^{2+}]_{er}$ was observed [124–128], thus raising the possibility that Bcl-2 dependent control of Ca^{2+} leakage may vary, for example, as a function of IP_3R subtype or phosphorylation. Interestingly, in these reports an inhibition of ER Ca^{2+} release kinetics was proposed, thus postulating, also in this case, reduced mitochondrial Ca^{2+} uptake [126,127]. Experiments with an ER-targeted mutant of Bcl-2 (Bcl-Cb5), in which the 21 C-terminal residues of Bcl-2 are replaced with the C-terminus of rat cytochrome *B5* [129], a TM protein that localizes to the ER [130] conclusively support the ER/mitochondrial Ca^{2+} link in apoptotic signaling. Bcl-Cb5 preserves mitochondrial function upon different apoptotic challenges, preventing the collapse of mitochondrial membrane potential [131] or the release of cytochrome *c* from mitochondria [132].

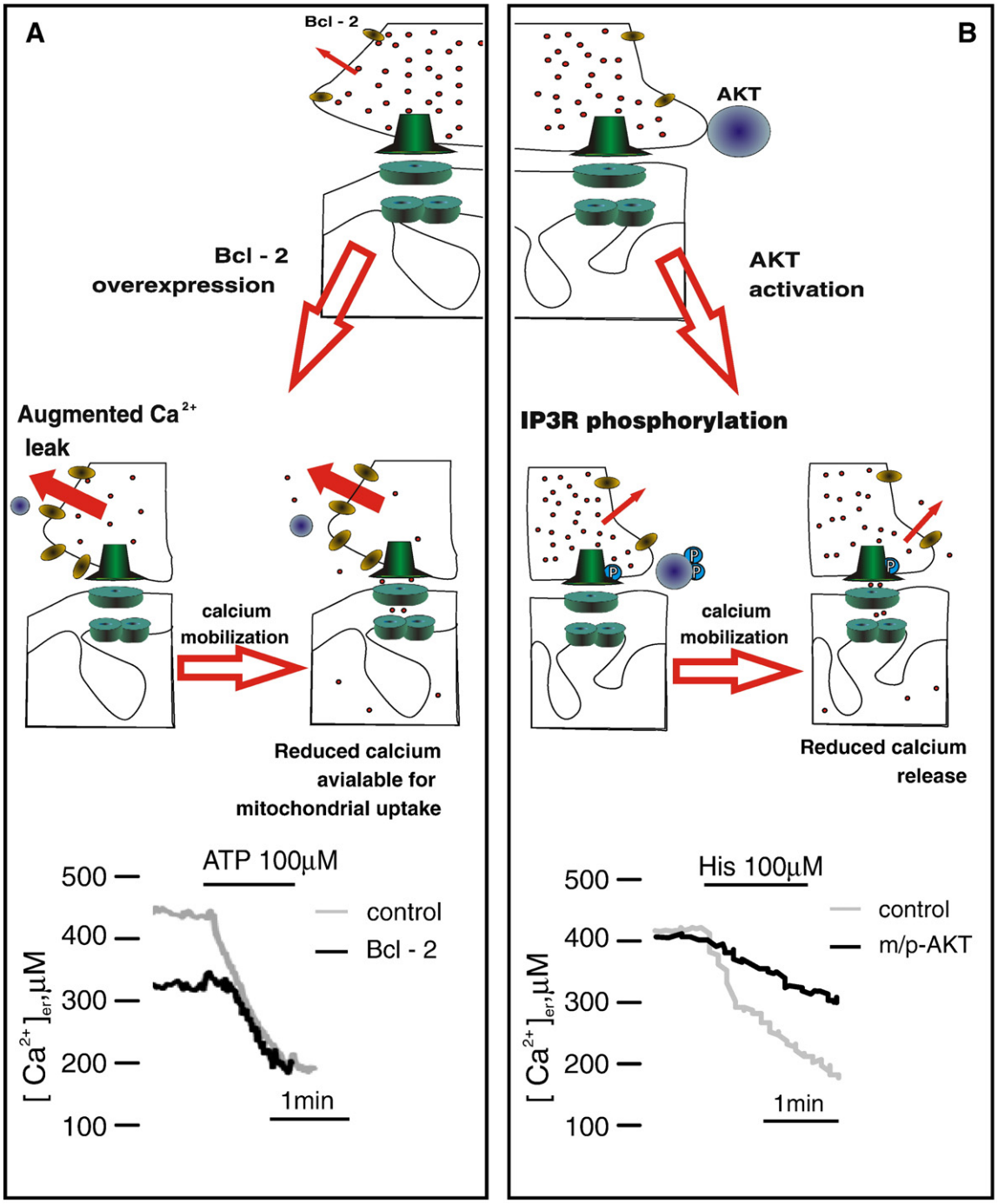

Fig. 2. Anti-apoptotic proteins Bcl-2 and Akt affect ER calcium homeostasis by differential mechanisms. (A) Bcl-2 overexpression increases the Ca^{2+} leak, while leaving Ca^{2+} accumulation unaffected, hence reducing the steady-state $[Ca^{2+}]$ levels. (B) Akt hyper-activation induces a decrease in ER Ca^{2+} release, probably through phosphorylation of IP_3R. As a direct consequence, the $[Ca^{2+}]$ increases caused by IP3-generating agonists were reduced in amplitude in both cytosol and mitochondria. Kinetics and $[Ca^{2+}]$ during agonist stimulation measured by ER-targeted aequorin in Bcl-2 overexpression or AKT hyper-activation conditions, are shown in the respective panels.

As to the events downstream of the ER/mitochondria Ca^{2+} cross-talk, the logical mechanism was sensitization of PTP, pore opening, mitochondrial morphological alterations and release of cytochrome *c*. Physiological Ca^{2+} loads were shown to sensitize PTP to apoptotic challenges [133] and reduction of Ca^{2+} signals by a variety of molecular and pharmacological approaches protected cells from apoptotic challenges [48]. The amount of releasable Ca^{2+}, rather than the $[Ca^{2+}]_{ER}$, seems to be the relevant parameter for the transduction of the death signal, as it controls the 'amplitude' of the signal reaching mitochondria. In agreement with this notion, over-expression of calreticulin (the most important luminal ER Ca^{2+} buffer) does not raise $[Ca^{2+}]_{er}$, but increases the amount of releasable Ca^{2+}, and cell survival is drastically reduced upon C2-ceramide treatment [48]. This result well matches the observation that cell lines derived from calreticulin knockouts are more resistant to apoptosis, indicating that the crucial requirement is the amount of Ca^{2+} released and not $[Ca^{2+}]_{er}$ [134].

Finally, it should be noted that, while thoroughly dissected for Bcl-2, this mechanism is likely to be shared by other anti-apoptotic proteins, not belonging to the Bcl-2 family. In support of this

possibility, we report the case of the Coxsackievirus 2b protein, an anti-apoptotic protein that extends the lifespan of the host cell by blocking caspase activation. By overexpressing the protein in HeLa cells, we showed that it lowers the filling state of the Ca^{2+} stores (ER and Golgi apparatus), affecting the Ca^{2+} transfer from ER to mitochondria [135].

6.2. Akt

Akt is an important sensor of the bioenergetics of the cell and therefore it is linked to the function of the mitochondria [136].

Akt is a serine/threonine kinase, which in mammals comprises three highly homologous members (PKB α, β and γ, also denominated Akt 1, 2 and 3) involved in the control of cellular processes as diverse as glucose metabolism, cell proliferation, apoptosis, transcription and cell migration. This enzyme has a key role in promoting cell survival utilizing different mechanisms that include the regulation of NF-κB activity and the inactivation of the pro-apoptotic protein Bad [137,138].

A few recent papers have drawn the attention on the possibility that a signaling target of Akt is the IP3R. In a recent paper, Joseph et al. reported IP_3R phosphorylation by Akt *in vitro* and *in vivo* in response to insulin activation of the PI-3 kinase pathway. The phosphorylation of the IP3R occurs in the C-terminal tail at a single consensus Akt phosphorylation site (serine 2618) that is present in all three IP_3R isoforms [57]. This is an interesting observation, because in some cancer cells in which Akt is constitutively active (e.g. prostatic carcinoma cells), IP_3Rs are hyper-phosphorylated. However, the authors report that phosphorylation does not alter the flux properties of the channel, even if it confers protection from apoptosis This observation is in contrast with previous work of the Roderick group [139], showing that phosphorylation of IP_3Rs by Akt inhibits Ca^{2+} release and apoptosis. Our own group has addressed this issue, by expressing a constitutively active form of Akt (myristoylated/palmytated Akt1, m/pAKT1) in HeLa cells and investigating Ca^{2+} homeostasis with targeted aequorins. Our results showed that m/p-AKT1 markedly inhibits Ca^{2+} release from IP_3R, after both agonist (e.g. histamine) and apoptotic stimuli (such as arachidonic acid and H_2O_2, two Ca^{2+}-dependent apoptotic stimuli). In turn, this alteration of ER Ca^{2+} release reduces significantly cellular sensitivity to Ca^{2+} mediated pro-apoptotic stimulation (Fig. 2B) [140]. Thus, these results appear coherent with those of Roderick et al., possibly suggesting that tissue-specific differences (e.g. expression or assembly of regulatory proteins) control the functional outcome of Akt-dependent phosphorylation.

7. Conclusions

A large body of works has followed the observation, almost two decades ago, that mitochondria undergo major swings in $[Ca^{2+}]$ upon cell stimulation. Two issues appear clear. The first is that this process has a major signaling function, as it participates in decoding cellular calcium signals in very different functional outcomes (e.g. regulation of cell metabolism or induction of cell death). The second is that, except for neurons, the swift response of the organelle depends on close contacts with the ER, and its Ca^{2+} channels. Research is rapidly progressing both on the identification of the targets of the Ca^{2+} signal, that are responsible for the downstream effects, and on the molecular machinery of the Ca^{2+} cross-talk between the two organelles (channels, scaffolding proteins, regulatory elements). We have reviewed the current knowledge on this topic (and the long-awaited issues to be solved), including the evidence that two important oncogenes (Bcl-2 and Akt) act on the ER/mitochondria Ca^{2+} transfer, further supporting the view that this cellular process may represent an interesting pharmacological target, with special reference to the development of novel anti-cancer drugs.

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References

[1] G. Hajnoczky, G. Csordas, M. Madesh, P. Pacher, The machinery of local Ca2+ signalling between sarco-endoplasmic reticulum and mitochondria, J. Physiol. 529 (Pt 1) (2000) 69–81.
[2] G. Hajnoczky, G. Csordas, M. Yi, Old players in a new role: mitochondria-associated membranes, VDAC, and ryanodine receptors as contributors to calcium signal propagation from endoplasmic reticulum to the mitochondria, Cell Calcium 32 (2002) 363–377.
[3] C. Mao, S.H. Kim, J.S. Almenoff, X.L. Rudner, D.M. Kearney, L.A. Kindman, Molecular cloning and characterization of SCaMPER, a sphingolipid Ca2+ release-mediating protein from endoplasmic reticulum, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 1993–1996.
[4] A. Galione, G.C. Churchill, Interactions between calcium release pathways: multiple messengers and multiple stores, Cell Calcium 32 (2002) 343–354.
[5] M. Oh-hora, A. Rao, Calcium signaling in lymphocytes, Curr. Opin. Immunol. 20 (2008) 250–258.
[6] M. Bertolino, R.R. Llinas, The central role of voltage-activated and receptor-operated calcium channels in neuronal cells, Annu. Rev. Pharmacol. Toxicol. 32 (1992) 399–421.
[7] I. McFadzean, A. Gibson, The developing relationship between receptor-operated and store-operated calcium channels in smooth muscle, Br. J. Pharmacol. 135 (2002) 1–13.
[8] J. Meldolesi, T. Pozzan, Pathways of Ca2+ influx at the plasma membrane: voltage-, receptor-, and second messenger-operated channels, Exp. Cell Res. 171 (1987) 271–283.
[9] P. Mitchell, Chemiosmotic coupling in oxidative and photosynthetic phosphorylation, Biol. Rev. Camb. Philos. Soc. 41 (1966) 445–502.
[10] R. Rizzuto, A.W. Simpson, M. Brini, T. Pozzan, Rapid changes of mitochondrial Ca2+ revealed by specifically targeted recombinant aequorin, Nature 358 (1992) 325–327.
[11] E. Rapizzi, P. Pinton, G. Szabadkai, M.R. Wieckowski, G. Vandecasteele, G. Baird, R.A. Tuft, K.E. Fogarty, R. Rizzuto, Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca2+ microdomains to mitochondria, J. Cell Biol. 159 (2002) 613–624.
[12] G. Csordas, M. Madesh, B. Antonsson, G. Hajnoczky, tcBid promotes Ca(2+) signal propagation to the mitochondria: control of Ca(2+) permeation through the outer mitochondrial membrane, EMBO J. 21 (2002) 2198–2206.
[13] V. Shoshan-Barmatz, D. Gincel, The voltage-dependent anion channel: characterization, modulation, and role in mitochondrial function in cell life and death, Cell Biochem. Biophys. 39 (2003) 279–292.
[14] S. Wu, M.J. Sampson, W.K. Decker, W.J. Craigen, Each mammalian mitochondrial outer membrane porin protein is dispensable: effects on cellular respiration, Biochim. Biophys. Acta 1452 (1999) 68–78.
[15] X. Xu, W. Decker, M.J. Sampson, W.J. Craigen, M. Colombini, Mouse VDAC isoforms expressed in yeast: channel properties and their roles in mitochondrial outer membrane permeability, J. Membr. Biol. 170 (1999) 89–102.
[16] M. Colombini, VDAC: the channel at the interface between mitochondria and the cytosol, Mol. Cell Biochem. 256–257 (2004) 107–115.
[17] A.C. Lee, X. Xu, M. Colombini, The role of pyridine dinucleotides in regulating the permeability of the mitochondrial outer membrane, J. Biol. Chem. 271 (1996) 26724–26731.
[18] M.G. Vander Heiden, X.X. Li, E. Gottleib, R.B. Hill, C.B. Thompson, M. Colombini, Bcl-xL promotes the open configuration of the voltage-dependent anion channel and metabolite passage through the outer mitochondrial membrane, J. Biol. Chem. 276 (2001) 19414–19419.
[19] J.G. Pastorino, J.B. Hoek, Regulation of hexokinase binding to VDAC, J. Bioenerg. Biomembr. 40 (2008) 171–182.
[20] C. Schwarzer, S. Barnikol-Watanabe, F.P. Thinnes, N. Hilschmann, Voltage-dependent anion-selective channel (VDAC) interacts with the dynein light chain Tctex1 and the heat-shock protein PBP74, Int. J. Biochem. Cell Biol. 34 (2002) 1059–1070.
[21] T.K. Rostovtseva, K.L. Sheldon, E. Hassanzadeh, C. Monge, V. Saks, S.M. Bezrukov, D.L. Sackett, Tubulin binding blocks mitochondrial voltage-dependent anion channel and regulates respiration, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 18746–18751.
[22] E.A. Jonas, J. Buchanan, L.K. Kaczmarek, Prolonged activation of mitochondrial conductances during synaptic transmission, Science 286 (1999) 1347–1350.
[23] T.K. Rostovtseva, W. Tan, M. Colombini, On the role of VDAC in apoptosis: fact and fiction, J. Bioenerg. Biomembr. 37 (2005) 129–142.
[24] W. Tan, M. Colombini, VDAC closure increases calcium ion flux, Biochim. Biophys. Acta 1768 (2007) 2510–2515.

[25] G. Szabadkai, K. Bianchi, P. Varnai, D. De Stefani, M.R. Wieckowski, D. Cavagna, A.I. Nagy, T. Balla, R. Rizzuto, Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels, J. Cell Biol. 175 (2006) 901–911.
[26] M.L. Litsky, D.R. Pfeiffer, Regulation of the mitochondrial Ca2+ uniporter by external adenine nucleotides: the uniporter behaves like a gated channel which is regulated by nucleotides and divalent cations, Biochemistry 36 (1997) 7071–7080.
[27] M. Montero, C.D. Lobaton, E. Hernandez-Sanmiguel, J. Santodomingo, L. Vay, A. Moreno, J. Alvarez, Direct activation of the mitochondrial calcium uniporter by natural plant flavonoids, Biochem. J. 384 (2004) 19–24.
[28] B. Moreau, C. Nelson, A.B. Parekh, Biphasic regulation of mitochondrial Ca2+ uptake by cytosolic Ca2+ concentration, Curr. Biol. 16 (2006) 1672–1677.
[29] C.D. Ferris, R.L. Huganir, D.S. Bredt, A.M. Cameron, S.H. Snyder, Inositol trisphosphate receptor: phosphorylation by protein kinase C and calcium calmodulin-dependent protein kinases in reconstituted lipid vesicles, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 2232–2235.
[30] P. Pinton, S. Leo, M.R. Wieckowski, G. Di Benedetto, R. Rizzuto, Long-term modulation of mitochondrial Ca2+ signals by protein kinase C isozymes, J. Cell Biol. 165 (2004) 223–232.
[31] M. Trenker, R. Malli, I. Fertschai, S. Levak-Frank, W.F. Graier, Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca2+ uniport, Nat. Cell Biol. 9 (2007) 445–452.
[32] P.S. Brookes, N. Parker, J.A. Buckingham, A. Vidal-Puig, A.P. Halestrap, T.E. Gunter, D.G. Nicholls, P. Bernardi, J.J. Lemasters, M.D. Brand, UCPs—unlikely calcium porters, Nat. Cell Biol. 10 (2008) 1235–1237 author reply 1237–1240.
[33] G.C. Sparagna, K.K. Gunter, S.S. Sheu, T.E. Gunter, Mitochondrial calcium uptake from physiological-type pulses of calcium. A description of the rapid uptake mode, J. Biol. Chem. 270 (1995) 27510–27515.
[34] T.E. Gunter, L. Buntinas, G. Sparagna, R. Eliseev, K. Gunter, Mitochondrial calcium transport: mechanisms and functions, Cell calcium 28 (2000) 285–296.
[35] G. Szalai, G. Csordas, B.M. Hantash, A.P. Thomas, G. Hajnoczky, Calcium signal transmission between ryanodine receptors and mitochondria, J. Biol. Chem. 275 (2000) 15305–15313.
[36] K. Baysal, D.W. Jung, K.K. Gunter, T.E. Gunter, G.P. Brierley, Na(+)-dependent Ca2+ efflux mechanism of heart mitochondria is not a passive Ca2+/2Na+ exchanger, Am. J. Physiol. 266 (1994) C800–808.
[37] D.R. Pfeiffer, T.E. Gunter, R. Eliseev, K.M. Broekemeier, K.K. Gunter, Release of Ca2+ from mitochondria via the saturable mechanisms and the permeability transition, IUBMB Life 52 (2001) 205–212.
[38] D.W. Jung, K. Baysal, G.P. Brierley, The sodium–calcium antiport of heart mitochondria is not electroneutral, J. Biol. Chem. 270 (1995) 672–678.
[39] H.Y. Chang, X. Yang, Proteases for cell suicide: functions and regulation of caspases, Microbiol. Mol. Biol. Rev. 64 (2000) 821–846.
[40] D.R. Hunter, R.A. Haworth, The Ca2+-induced membrane transition in mitochondria. I. The protective mechanisms, Arch. Biochem. Biophys. 195 (1979) 453–459.
[41] J.E. Kokoszka, K.G. Waymire, S.E. Levy, J.E. Sligh, J. Cai, D.P. Jones, G.R. MacGregor, D.C. Wallace, The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore, Nature 427 (2004) 461–465.
[42] A.P. Halestrap, P.M. Kerr, S. Javadov, K.Y. Woodfield, Elucidating the molecular mechanism of the permeability transition pore and its role in reperfusion injury of the heart, Biochim. Biophys. Acta 1366 (1998) 79–94.
[43] C.P. Baines, R.A. Kaiser, N.H. Purcell, N.S. Blair, H. Osinska, M.A. Hambleton, E.W. Brunskill, M.R. Sayen, R.A. Gottlieb, G.W. Dorn, J. Robbins, J.D. Molkentin, Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death, Nature 434 (2005) 658–662.
[44] E. Basso, L. Fante, J. Fowlkes, V. Petronilli, M.A. Forte, P. Bernardi, Properties of the permeability transition pore in mitochondria devoid of cyclophilin D, J. Biol. Chem. 280 (2005) 18558–18561.
[45] T. Nakagawa, S. Shimizu, T. Watanabe, O. Yamaguchi, K. Otsu, H. Yamagata, H. Inohara, T. Kubo, Y. Tsujimoto, Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death, Nature 434 (2005) 652–658.
[46] A.C. Schinzel, O. Takeuchi, Z. Huang, J.K. Fisher, Z. Zhou, J. Rubens, C. Hetz, N.N. Danial, M.A. Moskowitz, S.J. Korsmeyer, Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 12005–12010.
[47] A. Nicolli, E. Basso, V. Petronilli, R.M. Wenger, P. Bernardi, Interactions of cyclophilin with the mitochondrial inner membrane and regulation of the permeability transition pore, and cyclosporin A-sensitive channel, J. Biol. Chem. 271 (1996) 2185–2192.
[48] P. Pinton, D. Ferrari, E. Rapizzi, F. Di Virgilio, T. Pozzan, R. Rizzuto, The Ca2+ 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.
[49] P. Bernardi, M. Forte, The mitochondrial permeability transition pore, Novartis Found Symp. 287 (2007) 157–164 discussion 164–159.
[50] A.W. Leung, A.P. Halestrap, Recent progress in elucidating the molecular mechanism of the mitochondrial permeability transition pore, Biochim. Biophys. Acta 1777 (2008) 946–952.
[51] T. Litjens, T. Nguyen, J. Castro, E.C. Aromataris, L. Jones, G.J. Barritt, G.Y. Rychkov, Phospholipase C-gamma1 is required for the activation of store-operated Ca2+ channels in liver cells, Biochem. J. 405 (2007) 269–276.
[52] M.J. Rebecchi, S.N. Pentyala, Structure, function, and control of phosphoinositide-specific phospholipase C, Physiol. Rev. 80 (2000) 1291–1335.
[53] T. Furuichi, S. Yoshikawa, A. Miyawaki, K. Wada, N. Maeda, K. Mikoshiba, Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P400, Nature 342 (1989) 32–38.
[54] G.A. Mignery, T.C. Sudhof, The ligand binding site and transduction mechanism in the inositol-1,4,5-triphosphate receptor, EMBO J. 9 (1990) 3893–3898.
[55] K. Mikoshiba, IP3 receptor/Ca2+ channel: from discovery to new signaling concepts, J. Neurochem. 102 (2007) 1426–1446.
[56] A.E. Bugrim, Regulation of Ca2+ release by cAMP-dependent protein kinase. A mechanism for agonist-specific calcium signaling? Cell calcium 25 (1999) 219–226.
[57] M.T. Khan, L. Wagner II, D.I. Yule, C. Bhanumathy, S.K. Joseph, Akt kinase phosphorylation of inositol 1,4,5-trisphosphate receptors, J. Biol. Chem. 281 (2006) 3731–3737.
[58] K.S. Murthy, H. Zhou, Selective phosphorylation of the IP3R-I in vivo by cGMP-dependent protein kinase in smooth muscle, Am. J. Physiol. Gastrointest. Liver Physiol. 284 (2003) G221–230.
[59] C. Bagni, L. Mannucci, C.G. Dotti, F. Amaldi, Chemical stimulation of synaptosomes modulates alpha-Ca2+/calmodulin-dependent protein kinase II mRNA association to polysomes, J. Neurosci. 20 (2000) RC76.
[60] E. Vermassen, R.A. Fissore, N. Nadif Kasri, V. Vanderheyden, G. Callewaert, L. Missiaen, J.B. Parys, H. De Smedt, Regulation of the phosphorylation of the inositol 1,4,5-trisphosphate receptor by protein kinase C, Biochem Biophys Res Commun 319 (2004) 888–893.
[61] T. Jayaraman, K. Ondrias, E. Ondriasova, A.R. Marks, Regulation of the inositol 1,4,5-trisphosphate receptor by tyrosine phosphorylation, Science 272 (1996) 1492–1494.
[62] T. Furuichi, K. Kohda, A. Miyawaki, K. Mikoshiba, Intracellular channels, Curr. Opin. Neurobiol. 4 (1994) 294–303.
[63] M. Iwai, Y. Tateishi, M. Hattori, A. Mizutani, T. Nakamura, A. Futatsugi, T. Inoue, T. Furuichi, T. Michikawa, K. Mikoshiba, Molecular cloning of mouse type 2 and type 3 inositol 1,4,5-trisphosphate receptors and identification of a novel type 2 receptor splice variant, J. Biol. Chem. 280 (2005) 10305–10317.
[64] R.J. Wojcikiewicz, TypeI , II, and III inositol 1,4,5-trisphosphate receptors are unequally susceptible to down-regulation and are expressed in markedly different proportions in different cell types, J. Biol. Chem. 270 (1995) 11678–11683.
[65] M. Iwai, T. Michikawa, I. Bosanac, M. Ikura, K. Mikoshiba, Molecular basis of the isoform-specific ligand-binding affinity of inositol 1,4,5-trisphosphate receptors, J. Biol. Chem. 282 (2007) 12755–12764.
[66] M. Iino, M. Endo, Calcium-dependent immediate feedback control of inositol 1,4,5-triphosphate-induced Ca2+ release, Nature 360 (1992) 76–78.
[67] D.O. Mak, S. McBride, J.K. Foskett, ATP regulation of recombinant type 3 inositol 1,4,5-trisphosphate receptor gating, J. Gen. Physiol. 117 (2001) 447–456.
[68] L. Missiaen, C.W. Taylor, M.J. Berridge, Luminal Ca2+ promoting spontaneous Ca2+ release from inositol trisphosphate-sensitive stores in rat hepatocytes, J. Physiol. 455 (1992) 623–640.
[69] D.L. Nunn, C.W. Taylor, Luminal Ca2+ increases the sensitivity of Ca2+ stores to inositol 1,4,5-trisphosphate, Mol. Pharmacol. 41 (1992) 115–119.
[70] 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.
[71] G. Hajnoczky, R. Hager, A.P. Thomas, Mitochondria suppress local feedback activation of inositol 1,4, 5-trisphosphate receptors by Ca2+, J. Biol. Chem. 274 (1999) 14157–14162.
[72] E. Boitier, R. Rea, M.R. Duchen, Mitochondria exert a negative feedback on the propagation of intracellular Ca2+ waves in rat cortical astrocytes, J. Cell Biol. 145 (1999) 795–808.
[73] B. Landolfi, S. Curci, L. Debellis, T. Pozzan, A.M. Hofer, Ca2+ 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.
[74] J.B. Smith, L. Smith, B.L. Higgins, Temperature and nucleotide dependence of calcium release by myo-inositol 1,4,5-trisphosphate in cultured vascular smooth muscle cells, J. Biol. Chem. 260 (1985) 14413–14416.
[75] I. Bezprozvanny, B.E. Ehrlich, ATP modulates the function of inositol 1,4,5-trisphosphate-gated channels at two sites, Neuron 10 (1993) 1175–1184.
[76] M. Iino, Effects of adenine nucleotides on inositol 1,4,5-trisphosphate-induced calcium release in vascular smooth muscle cells, J. Gen. Physiol. 98 (1991) 681–698.
[77] Y. Tateishi, M. Hattori, T. Nakayama, M. Iwai, H. Bannai, T. Nakamura, T. Michikawa, T. Inoue, K. Mikoshiba, Cluster formation of inositol 1,4,5-trisphosphate receptor requires its transition to open state, J. Biol. Chem. 280 (2005) 6816–6822.
[78] I. Parker, I. Ivorra, Localized all-or-none calcium liberation by inositol trisphosphate, Science 250 (1990) 977–979.
[79] Y. Yao, J. Choi, I. Parker, Quantal puffs of intracellular Ca2+ evoked by inositol trisphosphate in *Xenopus* oocytes, J. Physiol. 482 (Pt 3) (1995) 533–553.
[80] T.A. Rooney, A.P. Thomas, Intracellular calcium waves generated by Ins(1,4,5)P3-dependent mechanisms, Cell Calcium 14 (1993) 674–690.
[81] N.L. Allbritton, T. Meyer, Localized calcium spikes and propagating calcium waves, Cell Calcium 14 (1993) 691–697.
[82] M.G. Lee, X. Xu, W. Zeng, J. Diaz, T.H. Kuo, F. Wuytack, L. Racymaekers, S. Muallem, Polarized expression of Ca2+ pumps in pancreatic and salivary gland cells. Role in initiation and propagation of [Ca2+]i waves, J. Biol. Chem. 272 (1997) 15771–15776.
[83] M.G. Lee, X. Xu, W. Zeng, J. Diaz, R.J. Wojcikiewicz, T.H. Kuo, F. Wuytack, L. Racymaekers, S. Muallem, Polarized expression of Ca2+ channels in pancreatic

and salivary gland cells. Correlation with initiation and propagation of [Ca2+]i waves, J. Biol. Chem. 272 (1997) 15765–15770.
[84] O.H. Petersen, D. Burdakov, A.V. Tepikin, Polarity in intracellular calcium signaling, Bioessays 21 (1999) 851–860.
[85] P. Thorn, A.M. Lawrie, P.M. Smith, D.V. Gallacher, O.H. Petersen, Local and global cytosolic Ca2+ oscillations in exocrine cells evoked by agonists and inositol trisphosphate, Cell 74 (1993) 661–668.
[86] O.H. Petersen, R. Sutton, Ca2+ signalling and pancreatitis: effects of alcohol, bile and coffee, Trends Pharmacol. Sci. 27 (2006) 113–120.
[87] 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 Ca2+ responses, Science 280 (1998) 1763–1766.
[88] G. Csordas, C. Renken, P. Varnai, L. Walter, D. Weaver, K.F. Buttle, T. Balla, C.A. Mannella, G. Hajnoczky, Structural and functional features and significance of the physical linkage between ER and mitochondria, J. Cell Biol. 174 (2006) 915–921.
[89] J.E. Vance, Phospholipid synthesis in a membrane fraction associated with mitochondria, J. Biol. Chem. 265 (1990) 7248–7256.
[90] J.E. Vance, Phosphatidylserine and phosphatidylethanolamine in mammalian cells: two metabolically related aminophospholipids, J. Lipid Res. 49 (2008) 1377–1387.
[91] S.S. Stone, M.C. Levin, P. Zhou, J. Han, T.C. Walther, R.V. Farese Jr., The endoplasmic reticulum enzyme, DGAT2, is found in mitochondria-associated membranes and has a mitochondrial targeting signal that promotes its association with mitochondria, J. Biol. Chem. 284 (8) (2009) 5352–5361.
[92] C. Garcia-Perez, G. Hajnoczky, G. Csordas, Physical coupling supports the local Ca2+ transfer between sarcoplasmic reticulum subdomains and the mitochondria in heart muscle, J. Biol. Chem. 283 (2008) 32771–32780.
[93] C. Ungermann, W. Neupert, D.M. Cyr, The role of Hsp70 in conferring unidirectionality on protein translocation into mitochondria, Science 266 (1994) 1250–1253.
[94] Q. Ran, R. Wadhwa, R. Kawai, S.C. Kaul, R.N. Sifers, R.J. Bick, J.R. Smith, O.M. Pereira-Smith, Extramitochondrial localization of mortalin/mthsp70/PBP74/GRP75, Biochem. Biophys. Res. Commun. 275 (2000) 174–179.
[95] T. Hayashi, T.P. Su, Sigma-1 receptor chaperones at the ER–mitochondrion interface regulate Ca(2+) signaling and cell survival, Cell 131 (2007) 596–610.
[96] T. Simmen, J.E. Aslan, A.D. Blagoveshchenskaya, L. Thomas, L. Wan, Y. Xiang, S.F. Feliciangeli, C.H. Hung, C.M. Crump, G. Thomas, PACS-2 controls endoplasmic reticulum–mitochondria communication and Bid-mediated apoptosis, EMBO J. 24 (2005) 717–729.
[97] P. Pacher, K. Sharma, G. Csordas, Y. Zhu, G. Hajnoczky, Uncoupling of ER–mitochondrial calcium communication by transforming growth factor-beta, Am. J. Physiol. Renal. Physiol. 295 (2008) F1303–F1312.
[98] M. Saotome, D. Safiulina, G. Szabadkai, S. Das, A. Fransson, P. Aspenstrom, R. Rizzuto, G. Hajnoczky, Bidirectional Ca2+-dependent control of mitochondrial dynamics by the Miro GTPase, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 20728–20733.
[99] M. Yi, D. Weaver, G. Hajnoczky, Control of mitochondrial motility and distribution by the calcium signal: a homeostatic circuit, J. Cell Biol. 167 (2004) 661–672.
[100] O.M. de Brito, L. Scorrano, Mitofusin 2 tethers endoplasmic reticulum to mitochondria, Nature 456 (2008) 605–610.
[101] J.G. McCormack, A.P. Halestrap, R.M. Denton, Role of calcium ions in regulation of mammalian intramitochondrial metabolism, Physiol. Rev. 70 (1990) 391–425.
[102] F.M. Lasorsa, P. Pinton, L. Palmieri, G. Fiermonte, R. Rizzuto, F. Palmieri, Recombinant expression of the Ca(2+)-sensitive aspartate/glutamate carrier increases mitochondrial ATP production in agonist-stimulated Chinese hamster ovary cells, J. Biol. Chem. 278 (2003) 38686–38692.
[103] C. Krieger, M.R. Duchen, Mitochondria, Ca2+ and neurodegenerative disease, Eur. J. Pharmacol. 447 (2002) 177–188.
[104] C. Giorgi, A. Romagnoli, P. Pinton, R. Rizzuto, Ca2+ signaling, mitochondria and cell death, Curr. Mol. Med. 8 (2008) 119–130.
[105] G. Szalai, R. Krishnamurthy, G. Hajnoczky, Apoptosis driven by IP(3)-linked mitochondrial calcium signals, EMBO J. 18 (1999) 6349–6361.
[106] C.C. Mendes, D.A. Gomes, M. Thompson, N.C. Souto, T.S. Goes, A.M. Goes, M.A. Rodrigues, M.V. Gomez, M.H. Nathanson, M.F. Leite, The type III inositol 1,4,5-trisphosphate receptor preferentially transmits apoptotic Ca2+ signals into mitochondria, J. Biol. Chem. 280 (2005) 40892–40900.
[107] T. Jayaraman, A.R. Marks, T cells deficient in inositol 1,4,5-trisphosphate receptor are resistant to apoptosis, Mol. Cell Biol. 17 (1997) 3005–3012.
[108] C. Steinmann, M.L. Landsverk, J.M. Barral, D. Boehning, Requirement of inositol 1,4,5-trisphosphate receptors for tumor-mediated lymphocyte apoptosis, J. Biol. Chem. 283 (2008) 13506–13509.
[109] Y. Tsujimoto, J. Yunis, L. Onorato-Showe, J. Erikson, P.C. Nowell, C.M. Croce, Molecular cloning of the chromosomal breakpoint of B-cell lymphomas and leukemias with the t(11;14) chromosome translocation, Science 224 (1984) 1403–1406.
[110] L. Pegoraro, A. Palumbo, J. Erikson, M. Falda, B. Giovanazzo, B.S. Emanuel, G. Rovera, P.C. Nowell, C.M. Croce, A 14;18 and an 8;14 chromosome translocation in a cell line derived from an acute B-cell leukemia, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 7166–7170.
[111] S. Cory, J.M. Adams, The Bcl2 family: regulators of the cellular life-or-death switch, Nat. Rev. Cancer 2 (2002) 647–656.
[112] Y. Akao, Y. Otsuki, S. Kataoka, Y. Ito, Y. Tsujimoto, Multiple subcellular localization of bcl-2: detection in nuclear outer membrane, endoplasmic reticulum membrane, and mitochondrial membranes, Cancer Res. 54 (1994) 2468–2471.
[113] S. Krajewski, S. Tanaka, S. Takayama, M.J. Schibler, W. Fenton, J.C. Reed, Investigation of the subcellular distribution of the bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes, Cancer Res. 53 (1993) 4701–4714.
[114] A.N. Murphy, D.E. Bredesen, G. Cortopassi, E. Wang, G. Fiskum, Bcl-2 potentiates the maximal calcium uptake capacity of neural cell mitochondria, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 9893–9898.
[115] J.C. Reed, H.S. Talwar, M. Cuddy, G. Baffy, J. Williamson, U.R. Rapp, G.J. Fisher, Mitochondrial protein p26 BCL2 reduces growth factor requirements of NIH3T3 fibroblasts, Exp. Cell Res. 195 (1991) 277–283.
[116] S.L. Schendel, M. Montal, J.C. Reed, Bcl-2 family proteins as ion-channels, Cell Death Differ 5 (1998) 372–380.
[117] P. Pinton, D. Ferrari, P. Magalhaes, K. Schulze-Osthoff, F. Di Virgilio, T. Pozzan, R. Rizzuto, Reduced loading of intracellular Ca(2+) stores and downregulation of capacitative Ca(2+) influx in Bcl-2-overexpressing cells, J. Cell Biol. 148 (2000) 857–862.
[118] M. Chami, A. Prandini, M. Campanella, P. Pinton, G. Szabadkai, J.C. Reed, R. Rizzuto, Bcl-2 and Bax exert opposing effects on Ca2+ signaling, which do not depend on their putative pore-forming region, J. Biol. Chem. 279 (2004) 54581–54589.
[119] C. White, C. Li, J. Yang, N.B. Petrenko, M. Madesh, C.B. Thompson, J.K. Foskett, The endoplasmic reticulum gateway to apoptosis by Bcl-X(L) modulation of the InsP3R, Nat. Cell Biol. 7 (2005) 1021–1028.
[120] R. Foyouzi-Youssefi, S. Arnaudeau, C. Borner, W.L. Kelley, J. Tschopp, D.P. Lew, N. Demaurex, K.H. Krause, Bcl-2 decreases the free Ca2+ concentration within the endoplasmic reticulum, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 5723–5728.
[121] A.E. Palmer, C. Jin, J.C. Reed, R.Y. Tsien, Bcl-2-mediated alterations in endoplasmic reticulum Ca2+ analyzed with an improved genetically encoded fluorescent sensor, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 17404–17409.
[122] L. Scorrano, S.A. Oakes, J.T. Opferman, E.H. Cheng, M.D. Sorcinelli, T. Pozzan, S.J. Korsmeyer, BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis, Science 300 (2003) 135–139.
[123] T.S. Ma, D.L. Mann, J.H. Lee, G.J. Gallinghouse, SR compartment calcium and cell apoptosis in SERCA overexpression, Cell Calcium 26 (1999) 25–36.
[124] R. Chen, I. Valencia, F. Zhong, K.S. McColl, H.L. Roderick, M.D. Bootman, M.J. Berridge, S.J. Conway, A.B. Holmes, G.A. Mignery, P. Velez, C.W. Distelhorst, Bcl-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5-trisphosphate, J. Cell Biol. 166 (2004) 193–203.
[125] C.W. Distelhorst, M. Lam, T.S. McCormick, Bcl-2 inhibits hydrogen peroxide-induced ER Ca2+ pool depletion, Oncogene 12 (1996) 2051–2055.
[126] H. He, M. Lam, T.S. McCormick, C.W. Distelhorst, Maintenance of calcium homeostasis in the endoplasmic reticulum by Bcl-2, J. Cell Biol. 138 (1997) 1219–1228.
[127] M. Lam, G. Dubyak, L. Chen, G. Nunez, R.L. Miesfeld, C.W. Distelhorst, Evidence that BCL-2 represses apoptosis by regulating endoplasmic reticulum-associated Ca2+ fluxes, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 6569–6573.
[128] N.S. Wang, M.T. Unkila, E.Z. Reineks, C.W. Distelhorst, Transient expression of wild-type or mitochondrially targeted Bcl-2 induces apoptosis, whereas transient expression of endoplasmic reticulum-targeted Bcl-2 is protective against Bax-induced cell death, J. Biol. Chem. 276 (2001) 44117–44128.
[129] W. Zhu, A. Cowie, G.W. Wasfy, L.Z. Penn, B. Leber, D.W. Andrews, Bcl-2 mutants with restricted subcellular location reveal spatially distinct pathways for apoptosis in different cell types, EMBO J. 15 (1996) 4130–4141.
[130] J. Mitoma, A. Ito, The carboxy-terminal 10 amino acid residues of cytochrome *b5* are necessary for its targeting to the endoplasmic reticulum, EMBO J. 11 (1992) 4197–4203.
[131] M.G. Annis, N. Zamzami, W. Zhu, L.Z. Penn, G. Kroemer, B. Leber, D.W. Andrews, Endoplasmic reticulum localized Bcl-2 prevents apoptosis when redistribution of cytochrome *c* is a late event, Oncogene 20 (2001) 1939–1952.
[132] J. Hacki, L. Egger, L. Monney, S. Conus, T. Rosse, I. Fellay, C. Borner, Apoptotic crosstalk between the endoplasmic reticulum and mitochondria controlled by Bcl-2, Oncogene 19 (2000) 2286–2295.
[133] P. Pacher, G. Hajnoczky, Propagation of the apoptotic signal by mitochondrial waves, Embo J. 20 (2001) 4107–4121.
[134] K. Nakamura, E. Bossy-Wetzel, K. Burns, M.P. Fadel, M. Lozyk, S. Goping, M. Opas, R.C. Bleackley, D.R. Green, M. Michalak, Changes in endoplasmic reticulum luminal environment affect cell sensitivity to apoptosis, J. Cell Biol. 150 (2000) 731–740.
[135] M. Campanella, A.S. de Jong, K.W. Lanke, W.J. Melchers, P.H. Willems, P. Pinton, R. Rizzuto, F.J. van Kuppeveld, The coxsackievirus 2B protein suppresses apoptotic host cell responses by manipulating intracellular Ca2+ homeostasis, J. Biol. Chem. 279 (2004) 18440–18450.
[136] B.D. Manning, L.C. Cantley, AKT/PKB signaling: navigating downstream, Cell 129 (2007) 1261–1274.
[137] D.P. Brazil, Z.Z. Yang, B.A. Hemmings, Advances in protein kinase B signalling: AKTion on multiple fronts, Trends Biochem. Sci. 29 (2004) 233–242.
[138] D. Kim, J. Chung, Akt: versatile mediator of cell survival and beyond, J. Biochem. Mol. Biol. 35 (2002) 106–115.
[139] T. Szado, V. Vanderheyden, J.B. Parys, H. De Smedt, K. Rietdorf, L. Kotelevets, E. Chastre, F. Khan, U. Landegren, O. Soderberg, M.D. Bootman, H.L. Roderick, Phosphorylation of inositol 1,4,5-trisphosphate receptors by protein kinase B/Akt inhibits Ca2+ release and apoptosis, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 2427–2432.
[140] S. Marchi, A. Rimessi, C. Giorgi, C. Baldini, L. Ferroni, R. Rizzuto, P. Pinton, Akt kinase reducing endoplasmic reticulum Ca2+ release protects cells from Ca2+-dependent apoptotic stimuli, Biochem. Biophys. Res. Commun. 375 (2008) 501–505.