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Mitochondria–endoplasmic reticulum choreography: structure and signaling dynamics

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Mitochondria and endoplasmic reticulum (ER) have different roles in living cells but they interact both physically and functionally. A key aspect of the mitochondria–ER relationship is the modulation of Ca^{2+} signaling during cell activation, which thus affects a variety of physiological processes. We focus here on the molecular aspects that control the dynamics of the organelle–organelle interaction and their relationship with Ca^{2+} signals, also discussing the consequences that these phenomena have, not only for cell physiology but also in the control of cell death.

Introduction

The presence of intracellular membrane-bound organelles is one of the defining features of eukaryotes compared with prokaryotes. In this review, we concentrate on the relationships between two of these organelles, the endoplasmic reticulum (ER) and mitochondria. Although their classical roles are distinct, evidence has accumulated pointing to the importance of their reciprocal structural and functional interactions for some key integrated cellular functions.

The best known role of mitochondria is that of the cell power station for ATP production, however, other key cell functions are also controlled by these organelles, such as lipid oxidation, oxygen radical production and hormone metabolism [1]. Over recent years, two additional functions of mitochondria have received particular attention: Ca^{2+} homeostasis and the release of apoptotic factors in response to death signals [2]. The ER is also involved in Ca^{2+} homeostasis because it acts as the principal internal store of Ca^{2+} ions. In addition, it is responsible for the synthesis of the majority of phospholipids and, in the case of the rough ER, of most membrane proteins and of soluble proteins destined to the secretory pathway. In striated muscle, the ER is organized structurally and functionally differently from that of other cells and is named sarcoplasmic reticulum (SR); the majority of the SR membrane network is devoid of bound ribosomes and is dedicated solely to the uptake and release of Ca^{2+}.

As discussed in more details in the following paragraphs, a major area of functional interaction between the ER and mitochondria is the control of Ca^{2+} signaling. This is a topic of major interest in physiology and pathology (for recent reviews, see [3]). Here, we summarize briefly a few general concepts. The $[Ca^{2+}]$ of the cytoplasm of all eukaryotic cells is maintained at very low levels under resting conditions (10–100 nM) and rapid increases ($\leq$1–2 μM) in cytoplasmic $[Ca^{2+}]$ are triggered either by Ca^{2+} release from the ER/SR and/or by Ca^{2+} influx through plasma membrane channels. The Ca^{2+} signal is then terminated through Ca^{2+} re-accumulation in the stores and/or extrusion into the external milieu. All cellular organelles participate in Ca^{2+} signaling, however, mitochondria that accumulate transiently (and then release) part of the Ca^{2+} coming from the ER/SR or through plasma membrane channels have a key role (see later). Owing to the high number of immobile Ca^{2+} buffers in the cytoplasm, the speed of Ca^{2+} diffusion is slow, $\sim$10 μm^2/sec and this enables the formation of intracellular gradients and microdomains (localized small cell regions in the proximity of Ca^{2+} channels) where the Ca^{2+} concentrations can transiently exceed the mean Ca^{2+} concentration of the bulk cytoplasm by several fold. Such microdomains have key roles in cell physiology; for example, for the release of neurotransmitters in synaptic terminals or for enabling fast mitochondrial uptake (reviewed in Ref. [4]). The Ca^{2+} signals are complex not only spatially but also temporally. Thus, in excitable cells, the Ca^{2+} increases follow the pattern of membrane-potential oscillations, however, in non-excitable cells, activation of membrane receptors also often results in repetitive cytosolic Ca^{2+} oscillations owing to cycles of Ca^{2+} release and uptake from the ER. Noteworthy, cells have devised ways to decode not only the amplitude but also the frequency of such Ca^{2+} oscillations [5,6].

In this review, we will focus on the structural and functional aspects of ER–mitochondria interactions that are pivotal in the control of Ca^{2+} signaling and of the processes that depend on them. In particular, as discussed in more detail later, physical interactions exist between the two organelles that might have a major role, both in the

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exchange of metabolites and in determining the efficiency of Ca^{2+} uptake by mitochondria in living cells, as well as in regulating the extent and speed of Ca^{2+} release from the ER/SR.

ER and mitochondria: structure and dynamics

The ER and mitochondria are known to be highly motile within most eukaryotic cells. Such dynamics depend on continuous fusion and budding of vesicles from the main membrane network (in the ER), fusion and fission between mitochondria and short- or long-range movements of individual vesicular elements along cytoskeletal structures. In a few cell types, such as striated muscle, the morphology of the two organelles is highly ordered and stable, characterized by the localization of part of the mitochondria in close apposition to SR terminal cysternae. The problem of SR–mitochondria interactions in striated muscle will not be dealt with here in any detail (but see [7] for more information).

The ER is a complex organelle composed of membrane sheets that enclose the nucleus (the nuclear envelope) and an elaborate interconnected tubular network in the cytosol. This highly dynamic and elaborate structure is maintained by a constant remodeling process that involves the formation of new tubules, their transport along the cytoskeleton and homotypic fusion [8]. The movement of ER tubules in animal cells is known to depend on the microtubule network, mainly reflecting microtubule dynamics and involving kinesin family proteins, although peripheral ER tubules also move along actin filaments [9]. Several proteins are involved in the interaction of polymerizing microtubules and ER structures [8]. These include p22, a myristoylated protein containing an EF-hand Ca^{2+}-binding domain; this protein binds microtubules and associates to ER membranes in a Ca^{2+}-dependent manner [10], providing a potential Ca^{2+}-regulated link between the ER and the cytoskeleton (see also later).

The classical view of mitochondria, that is, small (<1 μm in size) vesicular structures scattered throughout the cytoplasm, has been challenged over recent decades by the demonstration that mitochondria are rather represented by organelles of different sizes, in equilibrium with a highly dynamic reticular network, where the shape and size of each individual organelle can vary dramatically [11]. The movement of mitochondria can be limited to local, short-range motility from morphological alterations of the mitochondrial network or migrations of the circular organelles across long distances (e.g. along axons). It is generally believed that the movement of mitochondria depends on, and occurs along, the microtubule network, typically mediated by the molecular motor proteins kinesin and dynein; the actin cytoskeleton is also involved, although its role is less defined [12] (see also Ref. [13] in this issue). How molecular motors control mitochondrial movement is not completely clear. Recent work in *Drosophila* identified two proteins that seem to be important for mitochondrial transport: Milton, a cytoplasmic protein that can be co-immunoprecipitated with kinesin heavy chains [14], and Miro, an integral protein of the outer mitochondrial membrane (OMM) [15]. Kinesin, Milton and Miro are thought to work together, with Milton responsible for the attachment of kinesin to the organelles through Miro [16].

An interesting and unique feature of mitochondria is that the distribution of these organelles seems to vary according to local energy requirements. For example, within neurons, mitochondria are more crowded at sites associated with high energy demands, such as presynaptic terminals, active growth cones and branches, nodes of Ranvier or regions of axonal protein synthesis [17]. Moreover, glutamate-induced cytosolic Ca^{2+} increases inhibit mitochondrial mobility in neurons and this was suggested to occur as a result of local ATP depletion [18]. It is, however, still unclear whether ATP itself or other signals, Ca^{2+} in particular, control this mitochondrial localization. For example, in HEK293 cells, maximal mitochondrial movement occurs at the resting level of cytosolic Ca^{2+}, whereas complete suppression of motility is observed at the peak of agonist-induced cytosolic Ca^{2+} oscillations [19]. These and other findings have led to the proposal of a model in which the temporal and spatial coordinated regulation of mitochondria movement and their subcellular distribution is controlled by the cytosolic concentration of both Ca^{2+} and ATP, ensuring the right amount of energy is available at the right time and place within the cell [20]. Of interest, the mitochondrial protein Miro contains two predicted EF-hand domains [21,22] and it might be speculated that, thanks to these domains, Miro might be involved in Ca^{2+}-dependent regulation of mitochondrial motility [23]. It should be stressed that mitochondrial localization might also be modulated in response to other physiological signals: for example, in dorsal root ganglion neurons, activation of TrkA receptors by NGF activates PI3K, which results in actin-dependent docking of mitochondria in axons [24].

ER–mitochondrial interactions

It has been known for a long time that, in electron micrographs of different cell types, mitochondria and ER cysternae are often in close contact but such images were considered to be largely artifacts of fixation. Experiments in living cells with the two organelles labeled by green-fluorescent protein (GFP) [25] and electron micrograph images of samples frozen quickly [26] have demonstrated conclusively that such physical interactions between the two organelles indeed exist. Recently, it has also been shown by electron tomography that ER and mitochondria are adjoined by tethers (10 nm at the smooth ER and 25 nm at the rough ER) and that coupling between these two organelles can be weakened and strengthened by demolition and enforcement of this inter-organellar protein linkage [27]. Some evidence supports the hypothesis that the movement of mitochondria might occur concomitantly and in synchrony with that of specific ER regions. The mechanism of this reciprocal organelle-docking remains unresolved but it has been proposed that it depends on the expression on both membranes of complementary proteins that link the two organelles together, possibly at specific sites (Figure 1). For example, the voltage-dependent anion channel (VDAC) of the OMM interacts physically with the inositol 1,4,5-trisphosphate receptor (Ins(1,4,5)P_3R) on the ER through the molecular chaperone grp75 [28]. It

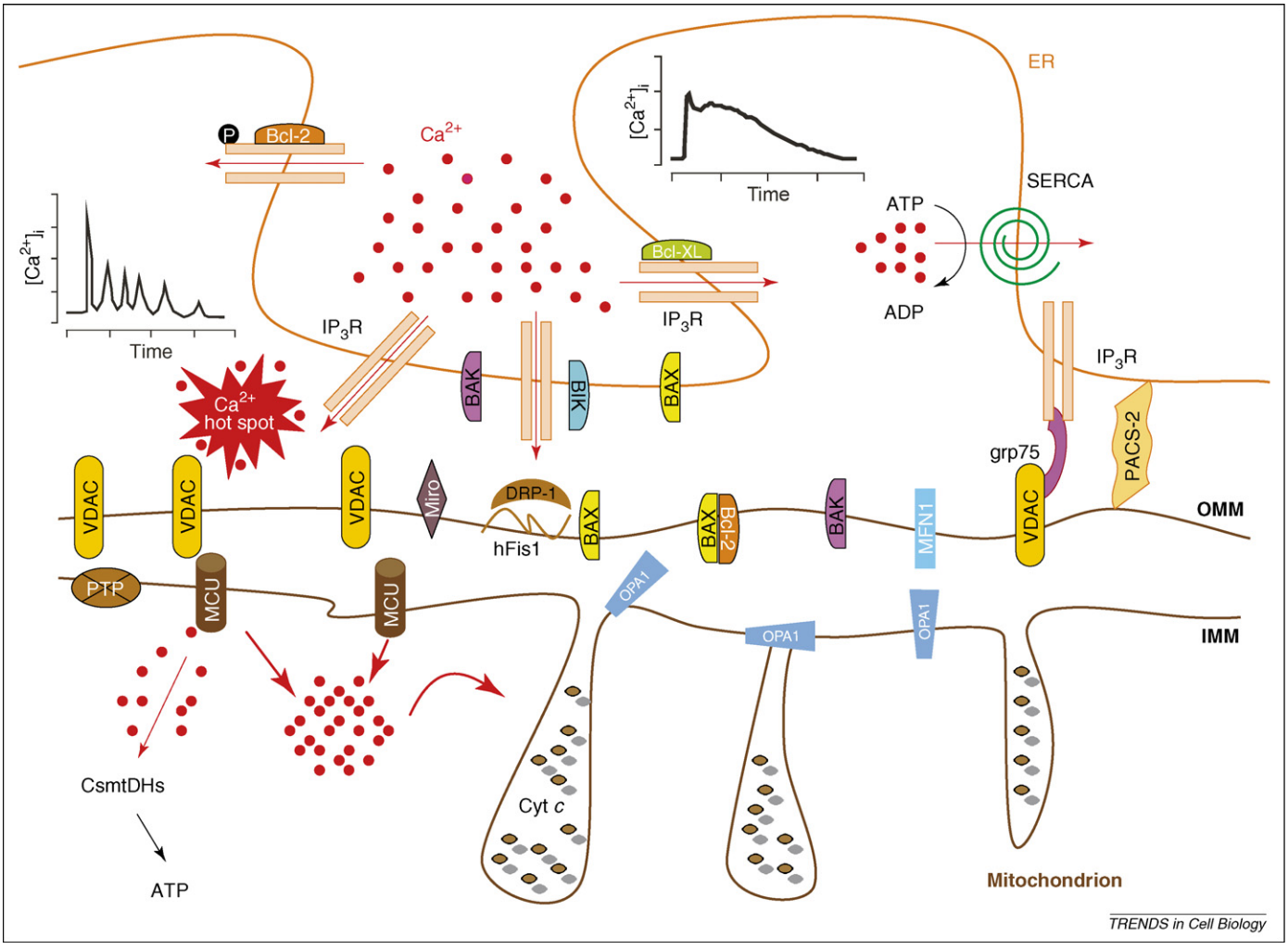

Figure 1. Local ER–mitochondria interactions. A representation is depicted of ER–mitochondria relationships and of some of the proteins involved in the cross-talk between the organelles. Ca^{2+} released from the ER through Ins(1,4,5)P_3Rs is taken up by mitochondria through the MCU. Within mitochondria, Ca^{2+} modulates mitochondrial dehydrogenase activity and thus ATP production. Moreover, mitochondrial Ca^{2+} uptake regulates the spatio-temporal pattern of the cytosolic Ca^{2+} signal and, therefore, many Ca^{2+}-dependent cellular processes. Massive and/or a prolonged accumulation of Ca^{2} in the mitochondria can lead to the opening of the PTP in the IMM and swelling of the organelle or, acting on mitochondria-shaping proteins [DRP-1, hFis1, OPA1, mitofusins (MFNs)], to mitochondrial cristae remodeling and modulation of apoptosis. The 'in concert' action of the two organelles in cell death is further supported by the presence on both the ER membrane and the OMM of different components of the Blc-2 family that have either a pro- (BIK, BAX, BAK) or an anti-apoptotic role (Bcl-2, Bcl-XL). ER and mitochondria could be linked physically at specific sites by cytoplasmic molecules (grp75, PACS-2), either directly or through proteins expressed on the two membranes (IP_3R, VDAC). Readers are referred to the text for further details. CsmtDHs, Ca^{2+} sensitive mitochondrial dehydrogenases; Cyt *c*, cytochrome *c*; IMM, inner mitochondrial membrane; IP_3R, inositol 1,4,5-trisphosphate receptor; OMM, outer mitochondrial membrane; MCU, mitochondrial Ca^{2+} uniporter; PTP, permeability transition pore; SERCA, sarcoplasmic-endoplasmic reticulum Ca^{2+} ATPase; VDAC, voltage-dependent anion channel.

is unknown presently whether and to what extent the physical interactions between the ER and mitochondria are stable or dynamic. However, it should be stressed that the close apposition of mitochondria to the ER membrane covers a small fraction of the organelle surface [25]. Accordingly, in the case of elongated mitochondria, only one part of the organelle is anchored to the ER and the rest can move independently.

The interactions between the two organelles seem to also be modulated by fission and fusion of mitochondria. Fission and fusion are regulated by a family of 'mitochondria-shaping proteins' (Figure 1). In mammals, the best characterized of these proteins is DRP-1 [29], a cytosolic dynamin-related protein that interacts at fission sites with its molecular adaptor hFis1 [30] (an integral protein of the OMM), and promotes mitochondrial fragmentation. Mitofusin (MFN)1 and 2 also reside in the OMM and regulate mitochondrial fusion [31]; the fusion mediated by MFN1 but not by MFN2 requires the inner mitochondrial membrane (IMM) protein OPA1 [32].

Fusion and fission of mitochondria can be regulated by Ca^{2+}. The recruitment of DRP-1 to mitochondria seems to be stimulated by Ca^{2+} uptake into the organelles [33], whereas fragmentation of the mitochondrial network, induced by over-production of DRP-1, reduces the global amplitude of mitochondrial Ca^{2+} responses [34]. Along the same lines, over-production of the ER-associated protein p20, a cleavage product of the Bcl-2-interacting protein BAP31, stimulates Ca^{2+} release from the ER and the subsequent recruitment of the fission protein DRP-1 to mitochondria [33]. Moreover, depletion of the multifunctional cytosolic-sorting protein PACS-2 has a similar effect, causing BAP31-dependent mitochondria fragmentation and uncoupling from the ER. In addition, PACS-2 regulates the formation of ER lipid-synthesizing centers as well as the translocation of the pro-apoptotic protein Bid to

mitochondria in response to apoptotic stimuli [35]. It has thus been suggested that PACS-2 could control the apposition of rod-like mitochondria to the ER, influencing multiple organelle-specific functions, such as mitochondrial fusion–fission events, lipid metabolism and apoptosis. The existence of mutual interactions between the two organelles is further supported by the finding that BIK, a BH3-only member of the Bcl-2 family, is anchored on the ER surface and induces the recruitment of DRP-1 to mitochondria [36] (Figure 1).

In summary, the processes regulating the morphology of the two organelles and their dynamic interactions have key functional consequences that will be analyzed in more detail in the following paragraphs. As to mitochondrial fusion and fission, this topic has been the subject of intense studies over recent years and the interested reader is referred to recent reviews for more detailed discussion (e.g. [37], or [38] in this issue).

ER–mitochondria interactions in cell signaling and metabolism

Exchange of metabolites between mitochondria and the ER occurs continuously during the life span of a cell. For example, ATP produced by oxidative phosphorylation is used by ATPases located within the ER (membrane or lumen); in the opposite direction, products of ER metabolic pathways, such as phospholipids, are transferred continuously to the mitochondrial membranes. As to ER–mitochondria phospholipid transfer, probably one of the best studied is that involved in phosphatidyletanolamine biosynthesis. Phosphatidylserine (PS) is, in fact, synthesized in the ER by two enzymes (phosphatidylserine synthase and phosphatidylethanolamine N-methyltransferase), transported to the mitochondria and decarboxylated at the outer surface of the IMM by phosphatidylserine decarboxylase [39]. This process of lipid transfer depends on the physical association between the two organelles. The transfer of phospholipids between the two organelles is consistent with a collision-based mechanism in which the two structures come into close contact with one another [40]. Indeed, a major fraction of PS is localized in an ER-related membrane fraction that is tightly associated to mitochondria, the so called 'mitochondria-associated membranes'.

However, the aspect of functional interaction between the ER and mitochondria that has received most attention in recent decades is undoubtedly that involving Ca^{2+} ions. This is because Ca^{2+} exchange between the ER and mitochondria not only has a crucial role in regulating the physical interactions between the two organelles, as described earlier, but it also regulates a variety of physiological processes, including aspects of apoptosis and necrosis.

The process of Ca^{2+} exchange between the ER and the mitochondria is often looked at simplistically as the ER being the Ca^{2+}-storage compartment releasing Ca^{2+} on stimulation and the mitochondria as the Ca^{2+} sinks that sequester part of the released cation. Although the uptake of Ca^{2+} released from the ER into the mitochondrial matrix is probably the best known and studied aspect of the crosstalk between the two organelles, Ca^{2+} on the cytosolic surface of both the ER and mitochondria can also have important regulatory roles. In particular: (i) the $[Ca^{2+}]$ in the intermembrane space (known to be indistinguishable from that of the surrounding cytosol) modulates the activity of metabolite carriers or of dehydrogenases located on the outer surface of the IMM; and (ii) the local mitochondrial Ca^{2+} sequestration has profound effects on the allosteric modulation by Ca^{2+} of the ER Ca^{2+}-release channels. As to the $[Ca^{2+}]$ within mitochondria, it is known to activate three key metabolic enzymes located in the matrix (pyruvate, α-ketoglutarate and isocitrate dehydrogenases).

We will now consider these three Ca^{2+} effects separately:

1. Two mitochondrial metabolite transporters, aralar1 and citrin, which are isoforms of the mitochondrial aspartate/glutamate carrier, are regulated by cytosolic Ca^{2+} thanks to the presence on their cytosolic tail of four EF-hand Ca^{2+}-binding motifs [41]. Similarly, the enzyme glycerol phosphate dehydrogenase, which is particularly active in insect flight muscle, but which is also a component of the redox shuttle whereby some mammalian tissues oxidize glycolytically derived NADH, is controlled by $[Ca^{2+}]$ in the intermembrane space [42]. Thus, Ca^{2+} released from the ER, even without entering the mitochondria, can participate in the modulation of mitochondrial metabolism and hence of ATP synthesis.
2. The allosteric modulation by Ca^{2+} of the two best known ER Ca^{2+}-release channels [the Ins(1,4,5)P_3R and the ryanodine receptor (RyR)] has been the subject of intense research over recent years and many excellent reviews have been published that deal with this topic [43,44]. Local $[Ca^{2+}]$ can have both a positive and a negative effect on the opening probability of these Ca^{2+} channels; mitochondria, by sequestering into their matrix part of the released Ca^{2+} might modify the cytosolic local Ca^{2+} level and thus the Ca^{2+} activation/inhibition of the ER channels themselves. Thus, resident mitochondria, especially acting as Ca^{2+} sinks in spatially restricted areas, could reduce the positive Ca^{2+} feedback on the Ins(1,4,5)P_3R (or RyR) and could also prevent the Ca^{2+}-dependent inactivation of the channels. In other words, depending on the tissue and the Ca^{2+}-release channel isoforms expressed, mitochondrial Ca^{2+} uptake can favor or reduce Ca^{2+} release from the ER (e.g. [45,46]).
3. Extensive literature has also been published on the role of intramitochondrial Ca^{2+} in the regulation of three matrix dehydrogenases (e.g. [47]). The best studied is the pyruvate dehydrogenase complex (Ca^{2+} activates a phosphatase, which in turn dephosphorylates three serine residues on the α-subunit of the enzyme, increasing the activity of the complex). The other Ca^{2+}-sensitive matrix dehydrogenases, NAD-isocitrate and the 2-oxoglutarate dehydrogenases, are activated by Ca^{2+} directly (reviewed in Ref. [42]). Thus, the overall result is that an increase in mitochondrial Ca^{2+} uptake, stimulated by ER Ca^{2+} release, activates ATP production, matching aerobic metabolism to energy demand (Figure 1). Direct measurement of mitochondrial ATP levels, by using a targeted chimera

of the ATP-sensitive photoprotein luciferase, indeed demonstrated directly that the Ca^{2+} rise within mitochondria enhances ATP production, an effect that lasts longer than the Ca^{2+} signal itself, highlighting a novel form of 'metabolic memory' at the cellular level [48].

This Ca^{2+}-dependent ER–mitochondria crosstalk in part depends on the capacity of mitochondria to accumulate Ca^{2+} within their matrix through the so-called 'Ca^{2+} uniporter'. This is a gated divalent cation channel [49] that drives Ca^{2+} into the mitochondrial matrix at the expense of the membrane potential, which is negative inside, generated by the respiratory chain (or by ATP hydrolysis). Ca^{2+} is then extruded back into the cytosol by antiporters (Na^+ or H^+/Ca^{2+}), driven by the ion gradients [4]. The molecular identities of the uniporter and of the antiporters are still unknown but a very recent report [50] has shed some light on the nature of the uniporter. In particular, it has been shown (using siRNA, cells from knockout mice and protein over-expression) that the so called 'uncoupling proteins 2 and 3' might be part of this elusive entity that has escaped identification, despite intense research, for over 40 years.

The speed and amplitude of Ca^{2+} accumulation in response to ER Ca^{2+} release in living cells are much faster and larger than expected from the low affinity for Ca^{2+} of the uniporter, as measured *in vitro* [51]. *In situ*, the rapid accumulation of Ca^{2+} by mitochondria seems to depend on the generation, close to the organelles, of cytosolic microdomains of high [Ca^{2+}] (Figure 1) due either to Ca^{2+} influx or ER Ca^{2+} release. Thus, in living cells, mitochondria respond promptly to physiological rises in cytosolic Ca^{2+} with very rapid and large increases in their matrix [Ca^{2+}], as shown clearly in different cell types (reviewed recently in Ref. [4]). Importantly, there seem not to be major kinetic limitations in the capacity of mitochondria to accumulate and release Ca^{2+} rapidly because fast uptake and release of Ca^{2+} from the organelles occurs in tissues, such as cardiac myoblasts [52] or skeletal muscle fibers [53], in which a single contraction–relaxation cycle is elicited by a cytosolic Ca^{2+} transient increase as short as 100–200 ms.

It should be stressed that, thanks to their Ca^{2+}-uptake capacity, mitochondria influence the spatio–temporal pattern of the Ca^{2+} signal within the cell and therefore many Ca^{2+}-dependent cellular processes. Indeed: (i) by buffering local Ca^{2+} and/or by returning it back to the cytosol after sequestration, mitochondria have a fundamental role in enabling the maintenance of cytosolic Ca^{2+} oscillations [54–56], (ii) by functioning as high-capacity Ca^{2+} sinks, they modulate the propagation of Ca^{2+} waves (e.g. in exocrine pancreas) [57], (iii) they reduce the amount of Ca^{2+} available to trigger exocytosis in adrenal cells [58] or (iv) they act in restricted microenvironments, modulating the behavior of specific Ca^{2+} channels [46,59–61].

A bidirectional Ca^{2+} coupling between mitochondria and the ER has been suggested to regulate other processes, such as thermogenesis in brown adipocytes [62] and aldosterone synthesis [63]. Finally, mitochondrial Ca^{2+} accumulation on cell stimulation could also have a long-lasting effect on cytoplasmic Ca^{2+} signals. Indeed Ca^{2+} release from loaded mitochondria might extend the duration of the cytosolic Ca^{2+} response, which could influence cell responses to subsequent stimuli [64]. For example, such a process can contribute to post-tetanic potentiation at the crayfish neuromuscular junction [65].

Mitochondria and ER cross-talk in cell death

Cell death, whether due to injury leading to necrosis or due to the tightly controlled process of apoptosis, has been the subject of a large number of studies over recent decades. It is firmly established that Ca^{2+} can have a major role in either form of cell death, as reviewed extensively recently (see [2,66]). Here, we limit ourselves to a few considerations linking the process of apoptosis to mitochondria–ER interactions.

The key process connecting apoptosis to the ER–mitochondria interactions is the discovery that a massive and/or a prolonged accumulation of Ca^{2+} into the mitochondria can lead to the opening of a large conductance pore in the IMM [the permeability transition pore (PTP)], swelling of the organelle, breakage of the OMM and release into the cytosol of a series of proapoptotic proteins, such as cytochrome *c*, apoptosis-inducing factor (AIF) and smac/diablo [67] (Box 1).

The role of the ER in supporting the mitochondrial apoptosis pathway is further demonstrated by several

Box 1. Interplay between ER and mitochondria in the regulation of apoptosis

A key step in the cell death signaling cascade involving mitochondria is the release from the inter-membrane space of several proapoptotic proteins, such as cytochrome *c*, apoptosis-inducing factor (AIF) and smac/diablo [67]. Loss of the outer mitochondrial membrane (OMM) permeability barrier might be dependent on the formation of specific pores (permeable to small proteins) made by the oligomerization on the OMM of proapoptotic proteins or on the rupture of the OMM, owing to swelling of the matrix. Swelling of the matrix depends on the activation of the so called 'permeability-transition pore' (PTP), a large nonselective channel (permeable to ions and solutes $\leq$1000–2000 Da) formed in the inner mitochondrial membrane (IMM) that causes massive ion and water influx into the matrix. The PTP can be opened by various conditions, including a prolonged Ca^{2+} influx. The Bcl-2 family includes more than 25 proteins that contain one or more Bcl-2 homology domain (BH domains). Functionally, Bcl-2s are divided into anti-apoptotic proteins, such as Bcl-2 and Bcl-X(L), and proapoptotic proteins, including Bax, Bak, Bid and Bad [77]. Bcl-2 proteins form oligomers with each other commonly and a delicate balance between anti- and pro-apoptotic proteins is required to maintain the integrity of the OMM permeability barrier. Bcl-2 members can either constitutively bind to both the ER and the mitochondrial membrane and/or are recruited from the cytoplasm onto the organelles on activation.

Some anti-apoptotic Bcl-2 members (Bcl-2 itself and Bcl-XL) can affect ER Ca^{2+} storage. For example, Bcl-2 over-expression results in a decrease of ER luminal [Ca^{2+}] and this effect has been attributed to an increase in the Ca^{2+} leak from the organelle [68–71]. Moreover, knockdown of Bax and Bak increases the interaction of Bcl-2 with the type-1 IP3Rs and promotes both the phosphorylation of the IP3R and a constitutive Ca^{2+} leak through the IP3Rs under basal conditions [72–74] (Figure 1). A reduced ER Ca^{2+} content, as in the case of Bcl-2 overexpression or knockdown of Bax/Bak, reduces the amount of Ca^{2+} that can be released from the ER owing to an apoptotic stimulus and thus decreases the probability of a Ca^{2+}-dependent PTP opening (reviewed recently in [76–79]). In addition, released Ca^{2+} from the ER, acting on mitochondria-shaping proteins (DRP-1, hFis1, OPA1, MFNs; Figure 1), can also influence mitochondrial cristae remodeling and thus the amount of cytochrome *c* that can be released in the cytosol [33].

findings, among which: (i) over-expression of anti-apoptotic proteins, such as Bcl–2, reduce the ER Ca^{2+} level [68–71]; (ii) down-regulation of proapoptotic proteins, such as Bak and Bax, reduces the ER Ca^{2+} concentration [72–74]; (iii) cytochrome *c* release linked to DRP-dependent mitochondrial fragmentation depends on ER Ca^{2+} release and BAP-31 cleavage [33,36] (Figure 1). It remains unclear how the rise in mitochondrial Ca^{2+} (that has probably evolved to couple cell signaling to metabolic activation) can be transformed into a trigger of cell death. Both the amplitude and, most importantly, the duration of the Ca^{2+} rise in the mitochondria have a major role in the transition. It is our biased opinion, however, that even more important to transform Ca^{2+} into a trigger of cell death is the concomitant production of other insults that affect mitochondrial functions (e.g. ceramide, oxygen radicals). In other words, the trigger for Ca^{2+}-dependent mitochondrial activation of cell death seems to be a form of coincidence detection system: only when both signals (Ca^{2+} and the toxic insult) are triggered simultaneously will the whole machinery of apoptosis become activated [75,76].

In the context of the mitochondria–ER relationship, it should be stressed that several proteins of the Bcl-2 family that have either a pro- or an anti-apoptotic role are located on both the ER membrane and the OMM, such as Bcl-2 itself, Bax, Bak and Bcl-XL (Figure 1), again indicating that the two organelles act in concert in this key process [77]. Space limitations prevent us from discussing further the involvement of mitochondria and ER Ca^{2+} in the control of apoptosis; the interested reader is referred to recent reviews [2,4,78,79] (Box 1).

Concluding remarks

The correct organization and the mutual interactions between the ER and mitochondria seem to coordinate important functions of the two organelles and to determine key aspects of cell physiology, death and survival. Future research in this field will be fundamental in understanding the molecular mechanisms that underlie specific ER–mitochondria communication in relation to the particular needs of different cells.

In particular, a few hot questions are still largely unanswered, such as: (i) what is the molecular nature of the proteins that link the ER and mitochondrial membranes? (ii) How are they regulated? (iii) What is the nature of the mitochondrial Ca^{2+} uniport and of the antiporters? We also expect that more quantitative data will become available on the size and concentration of the Ca^{2+} microdomains generated at the mitochondria–ER interface, on the mechanisms, regulation and role of mitochondrial fusion and fission and on the role of mitochondria/ER Ca^{2+}-dependent crosstalk in the control of apoptosis.

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