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Mitochondria and Endoplasmic Reticulum: The Lethal Interorganelle Cross-Talk

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The fundamental contribution of the mitochondria and ER to the decision made on the cell's fate has been increasingly recognized. This progress has illuminated the need for the mechanisms these organelles use to initiate and to propagate apoptotic signals. The toolbox of the mitochondria and ER is evolutionary conserved, overlapping and complementary. Furthermore, mitochondria are often closely associated with the ER providing the conditions for a local and privileged communication between the two organelles. The present review is concerned with the spatially and temporally coordinated utilization of Bcl-2 family proteins and Ca^{2+} by the mitochondria and ER to control the membrane permeabilization in the mitochondria and to regulate Ca^{2+} distribution and the activity of apoptotic proteins in the ER. The apoptotic means of the mitochondria and ER will eventually come together to control the dismantling of the cell by the caspases and other enzymes.

KEY WORDS: Mitochondria; ER; calcium; apoptosis; Bcl-2; Bax; PTP; IP_3R.

INTRODUCTION

The means used by the cell to determine its own destiny have always intrigued many researchers and have been a subject of passionate debates. The dynamics of research during the last 20 years, on the role of different intracellular organelles in cell death is illustrated by the number of publications in Fig. 1. For a long time, the nuclear chromatin was envisioned to serve as the site where the cell's fate is decided (hollow circles). In the nineties, evidence came as a surprise that the mitochondrion, an organelle well known about its functions in oxidative phosphorylation, plays a critical role in determining the destiny of the cell (black circles). Specifically, mitochondria were shown to release soluble factors, like cytochrome *c* (Liu *et al.*, 1996), which are sufficient alone to trigger a cell death executing mechanism in the cytosol or in the nucleus. Recent studies attract attention to the endoplasmic reticulum (ER), one of the most important intracellular calcium stores, as another organelle controlling the cell's fate (gray circles). In fact, the ER seems to be able to initiate a death program on its own but may also recruit the mitochondria to the execution cascade.

Certain inducers of programmed cell death (apoptosis) target directly the nucleus, the mitochondria, or the ER (Fig. 2 and reviewed in Ferri and Kroemer, 2001). Other factors engage their receptors in the cytosol or in the plasma membrane, which in turn, may also relay the signal to the nucleus, to the mitochondria or to the ER (Fig. 2). For example, cytosolic initiation commonly occurs through the modification and translocation of Bcl-2 family proteins (Bcl-2s) to the mitochondria, ER or other organelles (intrinsic pathway, Figs. 2–3). Plasma membrane initiation sites are the death receptors that mediate the effect of TNFα and Fas ligand to pro-caspase-8 activation (extrinsic pathway, Fig. 2). Depending on the cell

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Abbreviations used: Bcl-2s, Bcl-2 family proteins; $[Ca^{2+}]_c$, cytosolic $[Ca^{2+}]$; $[Ca^{2+}]_m$, mitochondrial matrix $[Ca^{2+}]$; CsA, cyclosporin A; ER, endoplasmic reticulum; IMM, inner mitochondrial membrane; IMS, inter membrane space; OMM, outer mitochondrial membrane; IP_3R, inositol 1,4,5-trisphosphate receptors; RyR, ryanodine receptors; PTP, permeability transition pore.

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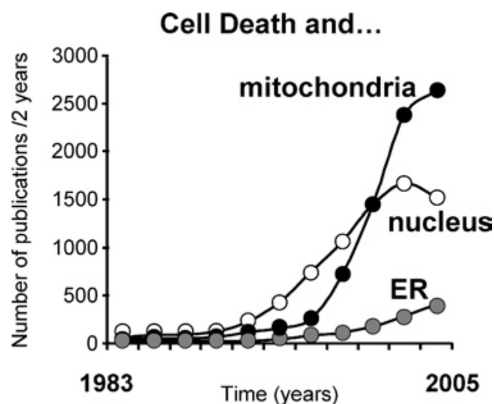


Fig. 1. Publications on the organelles and cell death in the past 20 years. The Pubmed data base was searched using the key words "nucleus and cell death" or "mitochondria and cell death" or "endoplasmic reticulum and cell death" in the period from April 1983 to April 2005. The number of the publications were calculated for every 2 years and plotted in the graph (nucleus: white symbols; mitochondria: black symbols and endoplasmic reticulum: gray symbols).

type, the activated caspase-8 either triggers the execution of a complex multi-caspase proteolytic cascade in the cytosol or through the cleavage of Bid or Bap31, it recruits the mitochondria and the ER to amplify the cell death signal. Thus, the mitochondria and the ER are important for both the initiation and propagation of the apoptotic signal.

Every initiation site described earlier is linked to the activation of the executioner caspase enzymes that are able to conduct the proteolytic cell destruction. This example illustrates that different initiation sites provide input to common execution pathways of apoptosis. However, to promote caspase activation, the plasma membrane death receptors recruit pro-caspase-8, the mitochondria undergo permeabilization and release intermembrane space (IMS) proteins (e.g., cytochrome *c*, Smac/Diablo and OMI/HtrA2), which promote the activation of cytosolic pro-caspase-9, while the ER initiation site seems to involve the ER membrane-targeted pro-caspase-12 in mice. Thus, different organelles render distinct mechanisms to activate common execution pathways. In addition, mitochondrial membrane permeabilization also provides some caspase-independent effectors (e.g., apoptotic inducing factor (AIF), endonuclease G), which seem to be specific for the mitochondrial pathway. Thus, there are execution mechanisms that are organelle specific.

Application of selective inhibitors and down-regulation/over-expression approaches permit the isolation of organelle specific death cascades. However the gateways to death are in complex interactions with each other, and in most paradigms of apoptosis, the death mechanisms of several organelles are recruited in a coordinated manner. The interactions between the organelles are mediated both by factors that seem to be specialized to control cell survival mechanisms (e.g., Bcl-2s) and by second messengers that control many aspects of cell function (e.g., Ca^{2+}).

The interconnected pathways make the apoptotic signal subtly regulated and allow the cell to control its destiny at several points. Since the ER and mitochondria have been shown to cooperate with each other in the control of physiological cell functions like metabolism and calcium signaling, and the significance of their interactions in apoptosis is an emerging topic of investigation, this review focuses on their relationship in the regulation of cell death pathways.

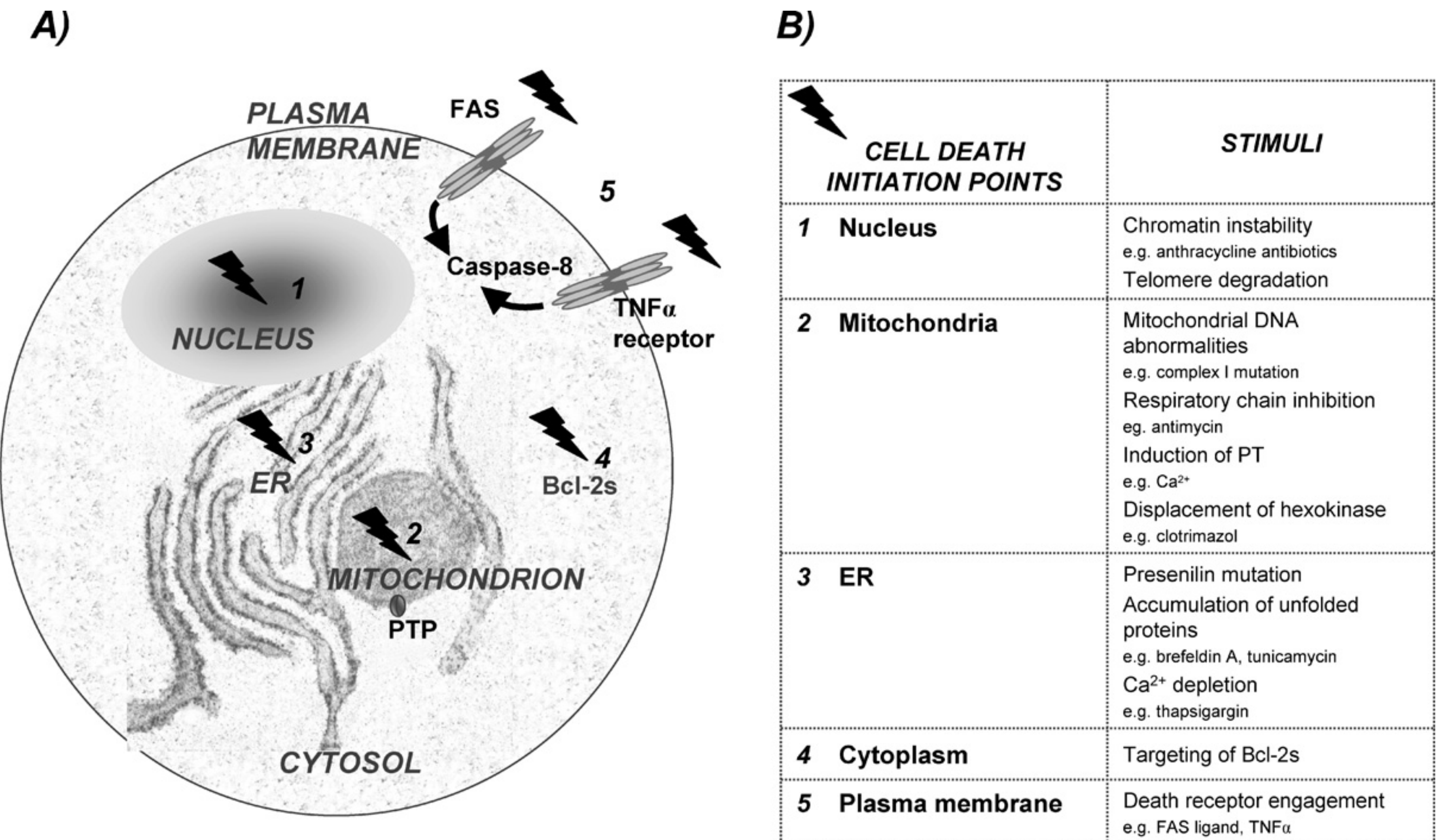
Mitochondria, as Initiator and Propagator of Death Signal

From the beginning of the aerobic life, mitochondria have always been a critical regulator of survival. Source of reactive oxygen species (ROS) by-products of the oxidative phosphorylation, protecting antioxidants, ATP and cytochrome *c*, the proto-mitochondrion inside the host cell may represent the foundation of the programmed cell death (reviewed in Blackstone and Green, 1999). The fact that Bcl-2 family protein machinery in bacteria, in worms and in mammals possesses the same features supports this hypothesis (reviewed in Reed *et al*., 2004).

Mitochondria have been implicated in the induction of cell death in a broad range of conditions, including anoxia/ischemia-reperfusion, hypoglycemia and neurodegenerative diseases and the list is growing every day. These data support the case of mitochondria as a focal organelle in cell pathophysiology (reviewed in Green and Kroemer, 2004). The most investigated and decisive step in the cell death cascade is the breakdown of the outer mitochondrial membrane (OMM), which opens a way for the release of several, pro-apoptotic proteins from the IMS to the cytosol (reviewed in Martinou and Green, 2001; Zamzami and Kroemer, 2001). Loss of the OMM barrier may be a consequence of the permeabilization of the inner mitochondrial membrane (IMM) mediated by the permeability transition pore (PTP). Alternatively, it may reflect selective permeabilization of the OMM induced commonly by apoptotic Bcl-2s.

Permeability Transition-PTP

Studies of the PTP started with the description of the permeability transition. The mitochondrial



CELL DEATH INITIATION POINTS	STIMULI
1 Nucleus	Chromatin instability e.g. anthracycline antibiotics Telomere degradation
2 Mitochondria	Mitochondrial DNA abnormalities e.g. complex I mutation Respiratory chain inhibition eg. antimycin Induction of PT e.g. Ca^{2+} Displacement of hexokinase e.g. clotrimazol
3 ER	Presenilin mutation Accumulation of unfolded proteins e.g. brefeldin A, tunicamycin Ca^{2+} depletion e.g. thapsigargin
4 Cytoplasm	Targeting of Bcl-2s
5 Plasma membrane	Death receptor engagement e.g. FAS ligand, TNFα

Fig. 2. Initiation points for cell death. (A) In a simplified scheme of the cell, an electron microscopy image shows a mitochondrion surrounded by the ER. The numbers mark five different initiation points for cell death: nucleus, mitochondrion, ER, cytosol and plasma membrane. (B) For each organelle a couple of examples of stimuli allowing initiation of a lethal message are described in the table.

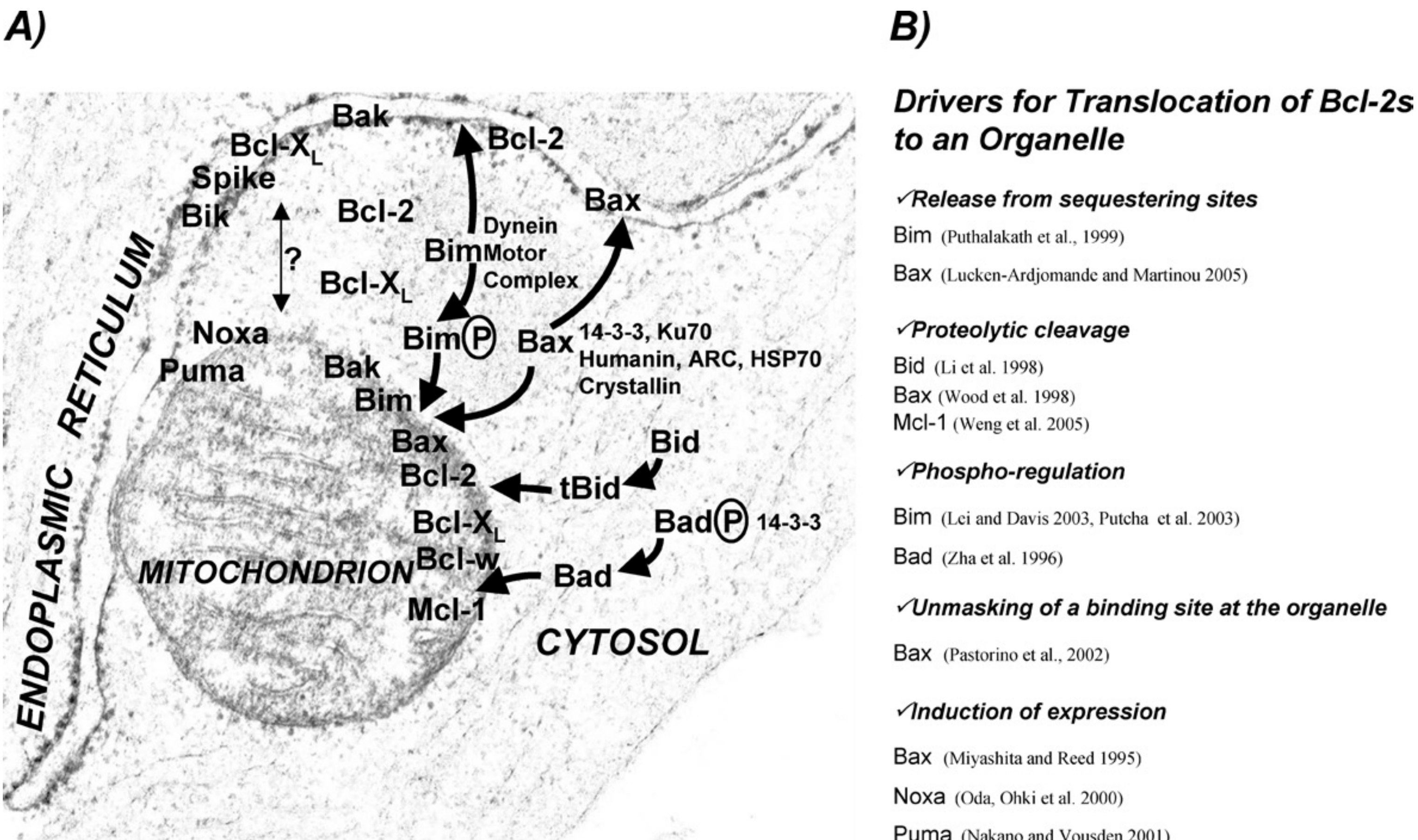


Fig. 3. Meeting points for Bcl-2s: mitochondria, ER, cytosol. (A) In the scheme, the distribution of Bcl-2s in the cytosol and in the mitochondrial and ER membranes is shown in surviving cells. Translocation of certain Bcl-2s from the cytosol to the organelles in response to stress stimuli are indicated by the arrows. (B) Different drivers controlling the translocation of the Bcl-2s from the cytosol to the membranes of the mitochondria or the ER during stress are listed in the table.

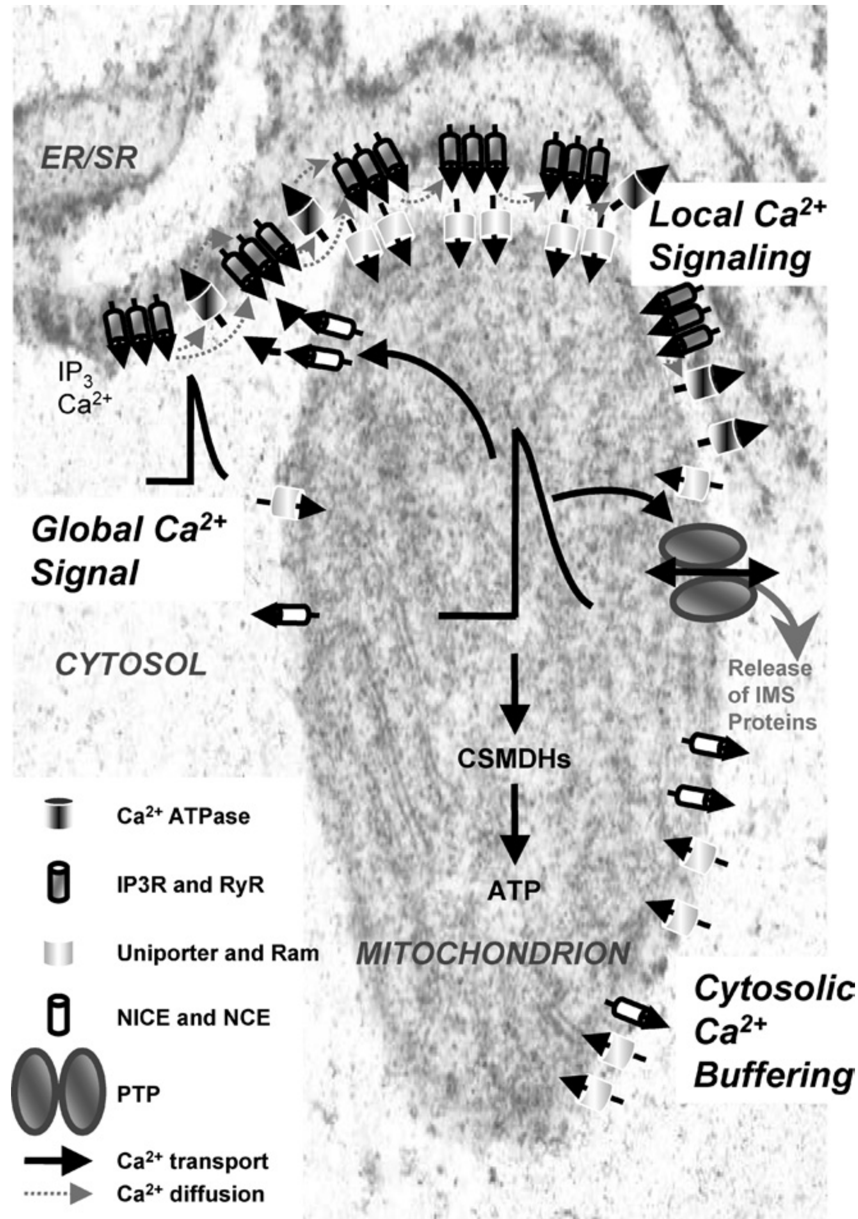


Fig. 4. Local and global aspects of the ER-mitochondrial Ca^{2+} signaling. The different mechanisms allowing uptake and release of Ca^{2+} by the mitochondria and ER are depicted in an electron microscopy image of an ER-mitochondrial association. Since a similar scheme applies to the sarcoplasmic reticulum (SR)-mitochondrial organization, the SR and the corresponding Ca^{2+} release channel, the ryanodine receptor (RyR) is also listed in the drawing. In the scheme, the Ca^{2+} transport through the OMM is not shown. The Ca^{2+} transport through the OMM is likely to be mediated by the VDAC. Abbreviations: CSMDH: Ca^{2+} sensitive mitochondrial dehydrogenases, NCE and NICE:Na^{+}-dependent and independent Ca^{2+} exchanger; Uniporter and Ram: Ca^{2+} uniporter and rapid uptake mode.

permeability transition was documented for the first time in the late 1970s (Hunter *et al.*, 1976; Haworth and Hunter, 1979; Hunter and Haworth, 1979a,b; Haworth and Hunter, 2000) in a series of papers, showing that mitochondria can undergo a regulated and reversible Ca^{2+}-induced swelling. Several years later, revelation of the pharmacologic control of the Ca^{2+}-induced swelling by cyclosporine A (CsA) promoted the consideration

of this phenomenon (Fournier *et al*., 1987; Crompton *et al*., 1988; Broekemeier *et al*., 1989). CsA was also shown to ameliorate the recovery after an ischemia-reperfusion event and to delay cell death (Halestrap *et al*., 1997). Recently, studies have provided genetic evidence for a fundamental role for the permeability transition in the Ca^{2+} and oxidative stress-induced cell death (Baines *et al*., 2005; Nakagawa *et al*., 2005).

The permeability transition is attributed to the opening of the PTP. The PTP is likely to be formed by a multi-protein complex, but its molecular nature remains a matter of controversy. One of the current models includes the VDAC/porin, an OMM channel, the adenine nucleotide translocator (ANT) that mediates the adenine nucleotide exchange across the IMM and the cyclophilin D, a matrix protein that binds CsA. However, it remains controversial whether these proteins are critical for the formation of the PTP and if they are the only components of the PTP. Regarding the ANT, the PTP opening is sensitive to the translocator ligands (adenine nucleotide depletion, bongkrekic acid, atractyloside (reviewed in Zoratti and Szabo, 1995) but is not eliminated in mitochondria lacking ANT (Kokoszka *et al*., 2004). Notably, targeting of cyclophilin D by CsA has been the most popular drug treatment to inhibit the PTP but the insensitivity to CsA does not necessarily exclude the lack of PTP opening (Bernardi and Petronilli, 1996). The sensitivity to CsA appears to be tissue dependent (Nicholls and Budd, 2000) and is lost when mitochondria are exposed to high doses of various PTP inducers (He and Lemasters, 2002). Furthermore, in mitochondria lacking cyclophilin D the Ca^{2+} sensitivity of the permeability transition is decreased but at higher Ca^{2+} loads, the PTP is formed (Basso *et al*., 2005).

The PTP is controlled endogenously by the mitochondrial matrix $[Ca^{2+}]$ ($[Ca^{2+}]_m$) but is also affected by the $\Delta\Psi_m$, by the redox state of the pyridine nucleotides, by the electron flux trough the first complex of the respiratory chain and by the ratio of ATP/ADP (reviewed in Zoratti and Szabo, 1995; Fontaine and Bernardi, 1999). The effects of these modulators can be integrated by the PTP and in fact, the ratio of the PTP inducers/PTP inhibitors determines the probability of the PTP opening. The presence of inducers may lower the threshold for the activation of the PTP by $[Ca^{2+}]_m$ and permit induction of the PTP during physiological $[Ca^{2+}]_m$ signals (Szalai *et al*., 1999). In addition to the changes in mitochondrial bioenergetics, protein-protein interactions emerge as important modulators of the PTP. The VDAC has been reported to directly interact with a variety of proteins (e.g., the peripheral benzodiazepine receptor (PBR) (McEnery *et al*., 1992), the ANT and the hexokinase (Beutner *et al*., 1996)) and several of these proteins are speculated to relay stress signals to the PTP. Binding of Bcl-2s to the putative components of the PTP have also been reported but the significance of these interactions in the gating of the PTP remains to be elucidated (see section "Interplay Between the PTP and Bcl-2s in the Mitochondria") In summary, the convergence of numerous regulatory mechanisms on the PTP indicates the significance of the PTP as a junction of cell death regulatory pathways.

Opening of the PTP permits equilibration of solutes ($\leq$1,500 daltons) across the IMM, which leads to an increase in the mitochondrial volume. Because the surface of the IMM is larger than the surface of the OMM, during swelling, rupture of the OMM occurs allowing the release of IMS proteins. In isolated mitochondria the PTP opening is commonly associated with large scale swelling but in intact cells, the presence of cytosolic proteins attenuates the swelling. In this case, opening of the PTP is reflected by the collapse of the $\Delta\Psi_m$ and release of stored Ca^{2+}. PTP-dependent release of IMS proteins has been shown in the absence of apparent mitochondrial swelling (Pacher and Hajnoczky, 2001), indicating that the PTP opening may activate an alternative mechanism of OMM permeabilization (De Giorgi *et al*., 2002). This may involve PTP-dependent recruitment of pro-apoptotic Bcl-2s to the mitochondria (De Giorgi *et al*., 2002) or the interaction of the PTP with previously recruited Bcl-2s (Madesh, Davies and Hajnoczky, unpublished data). In these phenomena, driving of the PTP by Ca^{2+} or pH transients results in transient PTP openings (Szalai *et al*., 1999; De Giorgi *et al*., 2002). This is important, because permanent permeabilization of the IMM switches mitochondria from energy production to energy consumption and moves the cells toward necrosis.

The opening of the PTP does not only provide an initiation point for the permeabilization for a given mitochondrion but it is also central to the recruitment of the entire population of mitochondria. Either electrical coupling (De Giorgi *et al*., 2000) or chemical coupling (regenerative Ca^{2+} release (Ichas *et al*., 1997; Pacher and Hajnoczky, 2001); reactive oxygen species (Zorov *et al*., 2000)) allow spreading of the membrane permeabilization among neighboring mitochondria. In summary, the PTP is a sensor for apoptotic stimuli and through mitochondrial membrane permeabilization initiates the execution of apoptosis. In addition, the PTP may receive input from other initiation sites and play a role in propagation and amplification of the apoptotic signal.

Selective OMM Permeabilization-Bcl-2-Dependent Pore

The Bcl-2 family includes more than 25 proteins that contain one ore more Bcl-2 homology domains (BH domains) and show similar fold structure. Functionally, Bcl-2s are divided to anti-apoptotic proteins including Bcl-2, Bcl-xL and Mcl-1 and pro-apoptotic proteins including Bax, Bak, Bid, Bad. The relevance of Bcl-2s for the control of cell death is supported by more than 10,000 research papers published on this topic. Cells deficient of both Bax and Bak are resistant to a wide range of apoptotic stimuli (Wei *et al*., 2001). The blockade of the death program appears at the release of the mitochondrial pro-apoptotic proteins, indicating that the Bcl-2s exert control over mitochondrial permeability and function.

In surviving cells, Bcl-2s localize both to the cytosol and to the membrane of the organelles (Fig. 3). Although Bcl-xL, Bcl-2, and Bax all form pores in artificial membranes, which permit the passage of $>$10,000 Da molecules, the OMM does not allow the release of IMS proteins and also limits the transport of small ions such as Ca^{2+} (Csordas *et al*., 2002; Rapizzi *et al*., 2002). However, if pro-apoptotic proteins redistribute from the cytosol or other organelles to the mitochondria (Fig. 3) or if the amount of anti-apoptotic Bcl-2s decreases, the OMM becomes permeable for large proteins. The permeabilization mechanism seems to involve the accumulation of Bax, Bak or Bax/Bak oligomers in the OMM (Mikhailov *et al*., 2003). Thus, a delicate balance between anti-and pro-apoptotic Bcl-2s in the OMM is required to maintain the integrity of the OMM barrier. Although, Bax can form channels in the absence of other proteins it can also bind to mitochondrial transporters (VDAC, ANT, see section "Interplay Between the PTP and Bcl-2s in the Mitochondria") and the exact composition of the pores formed in the OMM prior to cytochrome *c* release remains elusive. Importantly, the pore has to provide a conduction pathway for a variety of proteins in the 10,000–100,000 daltons range. Recent electrophysiology studies have documented the appearance of a Bax-containing channel, the mitochondrial apoptosis-induced channel (MAC) at the onset of apoptosis (Dejean *et al*., 2005). Based on the largest conductance level, this channel is estimated to provide for the permeation of cytochrome *c* and some larger proteins.

Recruitment of pro-apoptotic Bcl-2s to the mitochondria utilizes several different mechanisms (Fig. 3). Detachment of hexokinase II may represent a mitochondrial inititiation event for the translocation of cytosolic Bax to the mitochondria and for cytochrome *c* release (Pastorino *et al*., 2002; Majewski *et al*., 2004). Opening of the PTP has also been reported to promote the redistribution of Bax to the mitochondria (De Giorgi *et al*., 2002). Several pro-apoptotic proteins are retained in the cytosol as components of multiprotein complexes (Bax: humanin, Ku70, crystalline, (reviewed in Lucken-Ardjomande and Martinou, 2005); Bad: 14-3-3 (Zha *et al*., 1996); Bim: dynein motor complex (Puthalakath *et al*., 1999)). To facilitate the translocation to the mitochondria, apoptotic stimuli may weaken the interaction of Bax/Bad/Bim with the cytosolic retention factors. This may occur because of a conformational change in either the proteins that form the retention sites or in the pro-apoptotic proteins. An example for the later mechanism is the dephosphorylation of Bad (Zha *et al*., 1996). Furthermore, full-length Bid is retained in the cytosol by itself but proteolytic cleavage by caspase-8 (Luo *et al*., 1998) or by granzyme B (Barry *et al*., 2000) yields truncated Bid (tBid) that shows translocation to the mitochondria and induces OMM permeabilization (Li *et al*., 1998). In addition, apoptotic stimuli may simply stimulate the expression of pro-apoptotic Bcl-2s to enhance the amount bound to the mitochondria [Bax (Miyashita and Reed, 1995); Noxa (Oda *et al*., 2000); PUMA (Nakano and Vousden, 2001); BIK (Mathai *et al*., 2002)].

Bcl-2s commonly form oligomers with each other and the balance between the amounts of anti-and pro-apoptotic proteins seems to control the formation of oligomers of pro-apoptotic proteins in the OMM (Sharpe *et al*., 2004). Upon Bax-translocation to the mitochondria, the increase in the amount of Bax promotes the formation of Bax/Bax or Bax/Bak oligomers. tBid and Bad may interact with the anti-apoptotic Bcl-2s in the OMM to attenuate the neutralization of Bax and Bak and in turn, to stimulate the formation of pro-apoptotic oligomers. Furthermore, studies carried out with purified proteins indicate that tBid may trigger formation of the Bax-dependent pore even in the absence of anti-apoptotic Bcl-2s (Oh *et al*., 2005). The Bax and Bak oligomers have been proposed to be directly responsible for the disruption of the OMM integrity by forming pores big enough to allow the cytochrome *c* release from the mitochondria. Importantly, if cytochrome *c* is added to compensate for the release or ATP is provided to support the $\Delta\Psi_m$ generation by the F1F0-ATPase, the $\Delta\Psi_m$ will be maintained during the Bax/tBid-induced OMM permeabilization (Madesh *et al*., 2002). These data provide evidence that while the OMM was permeabilized the IMM barrier function was preserved. Thus, the translocation of Bcl-2s to the mitochondria and the interactions between anti-and pro-apoptotic Bcl-2s, where anti-apoptotic proteins have the capability to bind and neutralize the pro-apoptotic ones represent two major sites of the regulation of the OMM permeabilization.

Interplay Between the PTP and Bcl-2s in the Mitochondria

If a cell death pathway appears to depend on either the PTP or a Bcl-2 family protein, a frequently asked question is whether the pathway is independent from the other one. In some paradigms either the PTP or the Bcl-2s is dispensable but it is becoming clear that numerous mechanisms couple the PTP with the Bcl-2s. Photodamage induced PTP opening has been shown to signal the redistribution of Bax from the cytosol to the mitochondria, where it mediates cytochrome *c* release (De Giorgi *et al*., 2002). Evidence has also been presented that the Bcl-2s interact with the putative components of the PTP [Bax with ANT (Marzo *et al*., 1998); Bax with VDAC (Shimizu *et al*., 2000) and Bak with VDAC2 (Cheng *et al*., 2003)]. The effect of these interactions on the opening of the PTP remains controversial (Pastorino *et al*., 1999 vs. De Marchi *et al*., 2004). Bad, another pro-apoptotic Bcl-2 family protein has been detected in a complex that involves glucokinase (Danial *et al*., 2003), another putative VDAC-interacting protein. Anti-apoptotic Bcl-2s may form heterooligomeric complexes with Bax/Bak or may also bind to the PTP to control the opening. Thus, the major pathways allowing the initiation and propagation of the cell death signal in the mitochondria seem to offer an array of interactions. Although the PTP and the Bcl-2s seem to dominate the mechanisms of mitochondrial membrane permeabilization, some other pathways may also exist. For example, the superoxide anion induces cytochrome *c* release from the mitochondria apparently independent of the permeability transition and of the Bcl-2 family but dependent on VDAC (Madesh and Hajnoczky, 2001).

ER, as Initiator and Propagator of Death Signal

Besides its function in the control of multiple cellular processes like protein trafficking and Ca^{2+} signaling, the ER has been known for a long time as a major site of cellular injury in ischemia-reperfusion, and in degenerative diseases associated with cell loss including stroke, Parkinson's disease, Alzheimer's disease, HIV-associated dementia. The ER is highly sensitive to perturbations of the Ca^{2+} homeostasis (Ca^{2+} pump inhibitors, Ca^{2+} ionophores), inhibition of the glycosylation of proteins (tunicamyin), resorption of the Golgi apparatus into the ER and inhibition of protein secretion (brefeldin A) or other conditions leading to accumulation of misfolded proteins and oxidative injury. The ER response to stress is conserved from yeast to mammalian cells (Welihinda *et al*., 1999). The specific signaling pathways (like the unfolded protein response, UPR) stimulate an increase in the transcription of ER-resident chaperons and a decrease in the general protein synthesis. During severe stress, the ER response also includes the induction of the expression of pro-apoptotic proteins (GADD). Thus, depending on the severity of the ER stress and cell environmental conditions, the UPR either leads to cell survival or to cell destruction via apoptosis. The defense mechanism involves a coordinated communication between the ER and the cytosol and/or nucleus but no real implication of the mitochondria has been demonstrated so far. However, the accumulation of evidence has just started for the function of the ER as a gateway to cell death and for the broad relevance of the ER-mitochondrial communication in the initiation and execution of the apoptotic program.

Toolbox of the ER for Apoptosis Regulation: Ca^{2+}, Caspase-12, BAP31, JAFRAC2 . . .

The ER, as the mitochondria, is a meeting point of several pro-and anti-apoptotic mechanisms that mediate the cell's response to stress situations. In this regard, the ER Ca^{2+} storage function is particularly significant. The ER possesses an ATP-dependent Ca^{2+} pump (SERCA) allowing Ca^{2+} accumulation in the lumen of the organelle and Ca^{2+} channels, the inositol 1,4,5-trisphosphate receptors (IP_3Rs) and the ryanodine receptors (RyRs) that mediate rapid release of the stored Ca^{2+} (Fig. 4). Ca^{2+} liberation from the ER provides for an increase in cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_c$) and the ER Ca^{2+} depletion activates Ca^{2+} entry to magnify the $[Ca^{2+}]_c$ rise leading to a robust $[Ca^{2+}]_c$ signal in some cell types. The $[Ca^{2+}]_c$ signal has a fundamental role in the control of almost every aspects of physiological cell function but amplification, prolongation or combination of the $[Ca^{2+}]_c$ signal with stress agents also represents a potent trigger of cell death. For the induction of apoptosis, discharge of the ER Ca^{2+} store, elevation of the $[Ca^{2+}]_c$ as well as propagation of the calcium signal to mitochondria has been claimed to be important. In sensing the decrease in ER luminal $[Ca^{2+}]$ calreticulin, a high capacity Ca^{2+} binding protein that plays a major role in the quality control of proteins has been implicated. However the pro- and anti-apoptotic effects of calreticulin overexpression and deficiency (Nakamura *et al*., 2000), respectively may also reflect changes in the $[Ca^{2+}]_c$ and $[Ca^{2+}]_m$ signal (Camacho and Lechleiter, 1995; Arnaudeau *et al*., 2002). To induce apoptosis, the $[Ca^{2+}]_c$ signal may activate Ca^{2+} sensitive cytosolic enzymes, which for example control the distribution and activity of Bcl-2s (e.g., calcineurin (Wang *et al*., 1999), calpain (Wood *et al*., 1998; Chen *et al*., 2001)) or modulate the expression of apoptosis regulatory

proteins (Mesaeli and Phillipson, 2004). Propagation of the $[Ca^{2+}]_c$ signal to the mitochondria may facilitate the mitochondrial membrane permeabilization through activation of the PTP (see under section "Permeability Transition-PTP") and based on a recent study, through mediating Drp1-dependent fission of the mitochondria (Germain *et al.*, 2005).

Caspase-12, a protease belonging to the caspase family, is localized at the cytosolic surface of the ER and is specifically activated under ER stress in mouse (Nakagawa *et al.*, 2000). Caspase-12 activation has been attributed to calpain-mediated proteolytic cleavage of procaspase-12 (Nakagawa *et al.*, 2000) but other factors like TRAF2 (Yoneda *et al.*, 2001) and ER localized Bak (Zong *et al.*, 2003) and Bim (Morishima *et al.*, 2004) may also be involved. Caspase-12 was not activated by pro-apoptotic agents that target the mitochondria but activated in pathways insensitive to the down-regulation of the cytosolic cytochrome *c* partners Apaf-1 (Rao *et al.*, 2002), indicating that caspase-12 activation is independent of the mitochondria. When the caspase-12 is implicated in the way to death, the ER appears to be sufficient to sense, initiate and propagate the message but the dismantling of the cell is executed by the cytosolic caspase enzymes. Significantly, the caspase-12^{-}/$^{-}$ mice were found to be resistant to amyloid-β-peptide-induced apoptosis in an Alzheimer's disease model (Nakagawa *et al.*, 2000). However, the question of whether or not a functional human caspase-12 exists remains open. It is also important to note that the inducers of the caspase-12 pathway (e.g., thapsigargin and tunicamycin) have also been shown to evoke cytochrome *c* release in other paradigms, providing evidence for the mitochondrial involvement in the apoptotic program (Koya *et al.*, 2000; Jimbo *et al.*, 2003). It has also been suggested that in human cells, the response to stress induced by tunicamycin or brefeldin A have two components, one is linked to the protein homeostasis damage and is mitochondria-independent and the second one is linked to the perturbations of the Ca^{2+} homeostasis and is mitochondria-dependent (Kitamura *et al.*, 2003).

Another pro-apoptotic protein found at the ER is Bap31, a transmembrane protein that binds to nascent membranes proteins in transit between ER and *cis*-Golgi in normal conditions and is cleaved by caspase-8 to yield a pro-apoptotic segment p20 (Ng *et al.*, 1997). A current model predicts that during ER stress, the cleavage of Bap31 is mediated by pro-caspase-8L that is peripherally associated with the cytosolic face of the ER and is recruited to form a complex with Bap31 and in turn, to become activated (Breckenridge *et al.*, 2002). P20 induces Ca^{2+} release from the ER, converging with the other Ca^{2+} releasing mechanisms of the ER apoptotic pathway (Breckenridge *et al.*, 2003a).

While the most common mechanism of the mitochondrial initiation and propagation of the apoptotic signal may be the release of cytochrome *c* and other IMS proteins, no similar mechanism has emerged for the ER in mammalian cells. However, in insect cells, the thioredoxin peroxidase Jafrac2 has been identified as an Inhibitors of Apoptosis Protein (IAP)-interacting protein. Jafrac2 is synthesized as a precursor protein with an *N*-terminal peptide that targets it to ER lumen. Before any other apoptotic or morphological changes detectable, Jafrac2 is released to the cytosol following brefeldin A or tunicamycin-induced an ER stress or UV-induced apoptosis. Once in the cytosol, Jafrac2 neutralizes IAPs to trigger caspase activation and cell death (Tenev *et al.*, 2002). ER stress-induced by Ca^{2+} depletion, tunicamycin or brefeldin A has also been shown to induce early detachment of the c-Abl tyrosine kinase from the ER membrane and redistribution to the mitochondria. Based on studies with a c-Abl knockout model, the c-Abl translocation to the mitochondria is critical for the cytochrome *c* release and for the cell death (Ito *et al.*, 2001) and see section "Ca^{2+}-Independent Means of the ER-Mitochondrial Communication"). Taken together, these data suggest that the apoptosis toolkit of the ER includes several means like release of Ca^{2+}, activation of surface associated enzymes and perhaps the release of luminal proteins. Among the tools, caspase-12 and Jafrac2 appear to feed directly the activation of the executioner caspases, whereas Ca^{2+}, Bap31 and c-Abl recruit the mitochondria to the propagation of the apoptotic signal.

Bcl-2s in the ER

Similar to the OMM, the ER membrane contains several multidomain Bcl-2s like Bcl-2, Bcl-xL, Bax and Bak. Furthermore, in response to ER stress, translocation of Bax (Zong *et al.*, 2003) and Bim (Morishima *et al.*, 2004) from cytosol to the ER has been demonstrated (Fig. 3). Similar to the mitochondria, in the ER, the ratio of the pro- and anti-apoptotic proteins seems to control the cell's fate. However, some divergence has been noted between ER and mitochondria in the specific BH3-only Bcl-2s each contains. In the ER, Bik (Germain *et al.*, 2002) and see section "Interplay Between the PTP and Bcl-2s in the Mitochondria") and Spike (Mund *et al.*, 2003) have been found. Also, no translocation of tBid to the ER has been documented (Fig. 3). While the Bcl-2s converge in the mechanisms of membrane permeabilization in the mitochondria, Bcl-2s seems to control each means in the ER's apoptotic toolbox like Ca^{2+} transport,

caspase-12 activation and Bap31 proteolysis. Using mutants allowing either a gain or a loss of function targeted specifically to the ER compartment, the capacity of the Bcl-2s to regulate the cell death signal at the ER level has been extensively documented.

The presence of Bcl-2 at the ER has been shown even before its role in apoptosis regulation has been proposed (Chen-Levy *et al.*, 1989). One of the striking effects of Bcl-2 (and Bcl-xL) is on the ER Ca^{2+} storage (Palmer *et al.*, 2004 and reviewed in Ferrari *et al.*, 2002; Breckenridge *et al.*, 2003b; Oakes *et al.*, 2003; Annis *et al.*, 2004; Distelhorst and Shore, 2004). Bcl-2 overexpression has been shown to result in a decrease of ER luminal $[Ca^{2+}]$ and a moderate decrease of the store depletion induced $[Ca^{2+}]_c$ signal in several paradigms. The Bcl-2-induced lowering of the ER luminal $[Ca^{2+}]$ was attributed to an increase in the Ca^{2+} leak from the ER. However, in other systems, no effect or a Bcl-2-dependent enhancement of the store depletion induced $[Ca^{2+}]_c$ signal was documented. The capacity of Bcl-2 to control the ER Ca^{2+} store was decreased by Bcl-2 phosphorylation and was in correlation with the binding of BH3 only Bcl-2s to Bcl-2 (Bassik *et al.*, 2004). In addition to forming oligomers with other Bcl-2s, Bcl-2 seems to physically interact with the IP_3Rs (Chen *et al.*, 2004; Oakes *et al.*, 2005). Overexpression of Bcl-2 has been reported to attenuate the IP_3R-mediated Ca^{2+} flux (Chen *et al.*, 2004) and overexpression of Bcl-xL was shown to decrease type 1 IP_3R expression and the IP_3-dependent Ca^{2+} release (Li *et al.*, 2002). However, knockdown of Bax and Bak was shown to increase the interaction of Bcl-2 with the type-1 IP_3Rs and to promote both the phosphorylation of the IP_3R and the Ca^{2+} flux through the IP_3Rs (Oakes *et al.*, 2005). One clue to the diverse results can be the dependency of the Ca^{2+} transport regulation on the post-translational modification of both Bcl-2 and the IP_3Rs (Bassik *et al.*, 2004; Oakes *et al.*, 2005). Importantly, the IP_3R serves as a molecular scaffold for tens of IP_3R-interacting proteins, including protein kinases and other enzymes. Although the exact mechanism of the Bcl-2 effect on the IP_3R remains to be elucidated, the evidence on the interaction between Bcl-2 and IP_3Rs (Chen *et al.*, 2004; Oakes *et al.*, 2005) and on the dependence of cell death on both ER Ca^{2+} release and IP_3R expression (Sugawara *et al.*, 1997; Assefa *et al.*, 2004) firmly supports the consideration of the Bcl-2-IP_3R coupling as a major checkpoint in the control of apoptosis. The significance of the ER Bcl-2/Bcl-xL in the inhibition of the caspase-12 (Hetz *et al.*, 2003) and Bap31 pathways has also been documented (Ng *et al.*, 1997; Mund *et al.*, 2003). Thus each means of apoptosis in the ER seems to be under the control of ER Bcl-2/Bcl-xL. Similar to the regulation of the IP_3R, the control of Bap31 activation has been shown to involve formation of a complex of ER Bcl-2 with Bap31 (Ng *et al.*, 1997).

Using a combination of immuno-electron microscopy, confocal imaging and sub-cellular fractionation, ~10% of BAX and ~15% of BAK was detected in association with the ER in mouse embryonic fibroblasts (Scorrano *et al.*, 2003). Furthermore, during ER stress evoked by thapsigargin or tunicamycin, accumulation of Bax and conformational changes of Bax and Bak in the ER have been demonstrated (Zong *et al.*, 2003 but different results in Morishima *et al.*, 2004). Fibroblasts lacking both Bax and Bak were resistant to ER stress-induced apoptosis, and ectopic expression of Bax restores the sensitivity of these double deficient cells. Thus, the recruitment of Bax to the ER and the activation of Bax and Bak are required for the ER initiation and propagation of the apoptotic signal. Bak also seems to have a role in the control of ER volume and structure (Klee and Pimentel-Muinos, 2005). Present evidence favors to a mechanism whereby the effect of Bax and Bak is mediated through Bcl-2 or Bcl-xL to the ER-specific apoptotic factors (Oakes *et al.*, 2005).

Bik, a BH3 domain pro-apoptotic protein, has also been found integrated in the ER membrane, facing the cytosolic side. The amount of ER Bik seems to be controlled at the level of expression by stress conditions like genotoxic stress and over-expression of E1A or p53 (Mathai *et al.*, 2002) but not by ER stress conditions. Bik expression stimulates ER Ca^{2+} depletion in a Bax-Bak dependent manner (Mathai *et al.*, 2005). Spike is also ER localized and has been proposed to promote apoptosis via inhibition of the formation of a complex between Bap31 and Bcl-xL (Mund *et al.*, 2003). Bim, another BH3 only Bcl-2 family protein is present in the cytosol and during treatment of C2C12 cells with tunicamycin, translocates to the ER (Morishima *et al.*, 2004). In this model, Bim translocation was an important step toward activation of caspase-12 (see section "Toolbox of the ER for Apoptosis Regulation: Ca^{2+}, Caspase-12, BAP31, JAFRAC2 . . .") and initiation of the ER stress-specific caspase cascade.

Collaboration of the Mitochondria with the ER in Cell Survival and Cell Death

In many cell types, the mitochondria and the ER show broad cytosolic distribution. As revealed by high resolution microscopy approaches, numerous mitochondria show close contacts with the ER throughout the cytosol (examples are shown in Figs. 2–4) or embedded in multilamellar ER stacks (Mannella *et al.*, 1998). It has been estimated that 5–20% of the mitochondrial surface is in close appositions to the ER in living HeLa cells

(Rizzuto *et al.*, 1998). High density of IP_3Rs has also been demonstrated at the ER side of the interface, indicating a specialized function of the contact regions (Mignery *et al.*, 1989; Simpson *et al.*, 1997). The close associations may be stabilized by physical links between the ER and mitochondrial membranes. The mitochondria and ER are often bound to the cytoskeleton and using molecular motors, display movements along the tracks provided by the cytoskeleton. Recently, we have shown that the mitochondrial movements are inhibited by Ca^{2+}, providing a mechanism that supports recruitment of the mitochondria to the subcellular sites where Ca^{2+} is released from the ER (Yi *et al.*, 2004). Thus, mitochondria may interact with the ER via the cytosol throughout the cells but the close associations between mitochondria and the ER and the dynamic recruitment of mitochondria to the sites of ER Ca^{2+} release also provide conditions for a local communication between subdomains of these organelles.

Role of the ER-Mitochondrial Interface in Cell Metabolism and Signaling

The first role of a local communication between ER and mitochondria has been revealed in the phosphatidyletanolamine biosynthesis. In the phosphatidylserine (PS) pathway, the PS is synthesized in the ER, transported to the mitochondria and decarboxylated at the outer surface of the IMM. A major fraction of the PS was localized to an ER-related membrane fraction that is tightly associated with the mitochondria and referred as the mitochondria associated membranes (MAM). Transfer of PS to the IMM is predicted to be mediated by membrane contact (Vance, 1988, 1990). Recently, the MAMs have also been implicated as a privileged site in ceramide metabolism (Bionda *et al.*, 2004). Since ceramide production has been shown to induce apoptosis in the mitochondria (Birbes *et al.*, 2001), the MAMs may also be relevant as an initiation site of the cell death program. Although direct evidence has not been found yet, compartmentalization of some ribosomes and the TOM at the ER-mitochondrial associations would permit translocation of the mitochondrial targeted proteins to start during the translation (Lithgow, 2000).

Direct measurements of $[Ca^{2+}]_m$ *in situ* have also revealed that IP_3R-linked $[Ca^{2+}]_c$ spikes and oscillations evoke $[Ca^{2+}]_m$ spikes and oscillations and lead to parallel redox oscillations that are mediated by the activation of the mitochondrial Ca^{2+} sensitive dehydrogenases (Fig. 4, Rizzuto *et al.*, 1993; Hajnoczky *et al.*, 1995). This result was not expected since the global $[Ca^{2+}]_c$ signal did not reach the $[Ca^{2+}]$ range where activation of the mitochondrial Ca^{2+} uptake occurred. However, several lines of evidence have emerged that concurrent activation of the IP_3Rs in the ER resulted in a robust local $[Ca^{2+}]_c$ rise to stimulate the adjacent mitochondrial Ca^{2+} uptake sites at the ER-mitochondrial interface (Fig. 4). During IP_3-induced Ca^{2+} mobilization, 20–40% of the released Ca^{2+} may enter the mitochondria (Pacher *et al.*, 2000). The associations between the ER and mitochondria seem to have a privileged role in the interorganelle signaling (Rizzuto *et al.*, 1998) similar to several aspects of the synaptic communication between the neurons (Csordas *et al.*, 1999). Notably, the efficient local signal transmission is complemented with communication between the organelles through the bulk cytosol, which may become particularly relevant in the case of sustained signals. For example, prolonged $[Ca^{2+}]_c$ elevations cause gradual sensitization of the mitochondrial Ca^{2+} uptake sites to activate the large capacity mitochondrial Ca^{2+} buffering.

Mutual Dependence of the ER-and Mitochondria-Driven Apoptotic Programs

Recent studies have also revealed the significance of the ER-mitochondrial collaboration in pathophysiological situations. For instance, when the ER is exposed to stress often a mitochondrial damage can also be observed and protection against only the mitochondrial damage can prevent cell death. vMIA, an exclusively mitochondrial viral apoptosis inhibitor has been shown to prevent OMM permeabilization in BJAB and Hela cells exposed to ER-specific toxins (Boya *et al.*, 2002). Although the mitochondrial membrane permeabilization and the cell death were delayed, the injury of the ER was unaffected. Conversely, staurosporine induces OMM permeabilization to initiate apoptosis. However, protection against the staurosporine-induced cell death was observed in cells lacking IP_3Rs (Assefa *et al.*, 2004) or overexpressing ER-targeted Bcl-2 (Thomenius *et al.*, 2003). Furthermore, in Bax and Bak knockout cells, the staurosporine-induced cell death could be restored only if the consequences of Bax depletion were corrected in both mitochondria and ER (Scorrano *et al.*, 2003). Thus, if the primary effect of staurosporine was confined to the mitochondria, the above results indicate that the propagation of the cell death program through the mitochondrial pathway was dependent on the utilization of some tools from the toolbox of the ER.

The pathway adopted to induce the cell's demise varies according to the cell type and the cell death inducers, making difficult to outline a generalized roadmap to cell death. However, organelle-specific targeting of the Bcl-2s and evaluation of the interaction with different cell death-inducing agents have helped to

sort out some patterns of the respective contribution of ER and mitochondria. In one of these studies, a Bcl-2 variant targeted exclusively to the ER was able to inhibit apoptosis induced by serum starvation or ceramide but not by etoposide in Rat-1 cells (Annis *et al*., 2001). Studying the sequence of the two mitochondrial events, namely the cytochrome *c* release and the loss of the $\Delta\Psi_m$, the authors found that the ER Bcl-2 was able to inhibit apoptosis only when the collapse of the $\Delta\Psi_m$ occurred before the cytochrome *c* release. Thus the ER contribution was more important when the IMM function was lost first. This situation might reflect initiation of apoptosis through the opening of the PTP. The lack of CsA sensitivity of the ceramide-induced cell death did not support this idea but other PTP inhibitors have to be also applied to test the opening of the PTP (see section "Permeability Transition-PTP"). A powerful array of genetic approaches was used by Scorrano *et al*. (Scorrano *et al*., 2003) to demonstrate that the tBid expression-induced cell death did not need Bax or Bak in the ER. This result is consistent with the previous study, since tBid has been shown to induce OMM permeabilization without any disintegration of the IMM (Madesh *et al*., 2002). Furthermore, the arachidonic acid-or H_2O_2-induced cell death required ER Bax or Bak. This cell death pathway could also be restored by overexpression of the SERCA pump in the Bax and Bak knockout cells. Thus, the ER Bax or Bak seems to contribute to the cell death program by an effect on the ER Ca^{2+} regulation. Notably, both the arachidonic acid and H_2O_2 induced cell death was found to be PTP-dependent in other cell types (Scorrano *et al*., 2001; Takeyama *et al*., 2002). Along this line, restoration of the ER Ca^{2+} store by the Bax or SERCA expression may be required to provide Ca^{2+}, a major inducer of the PTP. Finally, in this study, the death induction by a third group of apoptotic stimuli, including staurosporine, etoposide and brefeldin A was restored only by reloading the ER with Ca^{2+} and mitochondria with Bax. Thus these agents either induce at one organelle a cascade that also recruits the other organelle or activate both the mitochondrial and ER pathways in parallel. Since ER targeted overexpression of Bcl-2 has been shown to prevent the cytochrome *c* release induced by staurosporine (Annis *et al*., 2001) and brefeldin A + cycloheximide (Hacki *et al*., 2000), the parallel pathways would need to interact upstream of the cytochrome *c* release. However, the results with the etoposide indicate different roles for the ER Bcl-2s in the different paradigms (Annis *et al*., 2001; Scorrano *et al*., 2003). In summary, recruitment of the ER does not seem to be important when apoptosis is induced by direct and selective targeting of the OMM (tBid) but in the case of many other inducers, the ER-mitochondrial communication offers a Bcl-2s dependent amplification mechanism of the death signal.

Ca^{2+} as the Principal Language of the ER-Mitochondrial Communication

To achieve a goal, a common language is necessary in any collaboration. What is the one used by the mitochondrion and the ER? Ca^{2+} seems to be the main component of the ER-mitochondrial dialogue for several reasons. Both the mitochondria and the ER have effective Ca^{2+} transport mechanisms to control both the internal $[Ca^{2+}]$ and the $[Ca^{2+}]_c$. In both ER and mitochondria, the control of the Ca^{2+} transport sites may give rise to regenerative phenomena (e.g., Ca^{2+}-induced Ca^{2+} release, Fig. 4), which permit propagation of the Ca^{2+} signal throughout the cells. The ER Ca^{2+} release sites, the IP_3Rs and RyRs interact with a variety of regulatory factors to establish sophisticated control of the channel's gating. However, the molecular identity and the regulation of the mitochondrial Ca^{2+} transporters remain elusive. Mitochondria and the ER maintain a local Ca^{2+} communication pathway that allows highly effective Ca^{2+} transfer between the two organelles. During Ca^{2+} mobilization from the ER, the mitochondria may accumulate Ca^{2+} even in the absence of a global $[Ca^{2+}]_c$ rise (Rizzuto *et al*., 1993; Csordas *et al*., 1999) and in some conditions of the PTP-mediated mitochondrial Ca^{2+} release the ER may take up the Ca^{2+} before it is detected in the cytosol (Bowser *et al*., 2002).

Ca^{2+} controls many aspects of the physiological function of each organelle and is a major component of the apoptotic repertoire of both the mitochondria and the ER. The most characterized target of Ca^{2+} in the mitochondria is the PTP (see section "Permeability Transition-PTP"). In some paradigms, the stress agents target the mitochondria and result in sensitization of the PTP towards activation by $[Ca^{2+}]_m$. Owing to the sensitization of the PTP, the delivery of physiological $[Ca^{2+}]_c$ oscillations to the mitochondria may become effective to induce PTP opening and in turn, apoptosis (Szalai *et al*., 1999). Alternatively, ER-targeted stress may activate an early Ca^{2+} release from the ER and store-operated Ca^{2+} entry and the surplus Ca^{2+} redistributes to the mitochondria. The efficacy of inhibitors of the mitochondrial Ca^{2+} uptake to suppress the cell death illustrates the pathophysiological significance of the mitochondrial Ca^{2+} uptake (Groskreutz *et al*., 1992; Dessi *et al*., 1995; Bae *et al*., 2003). In addition, the restoration of the arachidonic acid and H_2O_2-induced cell death in Bax and Bak knockout cells with overexpression of the SERCA (see Mutual Dependence of the ER-and Mitochondria-Driven Apoptotic Programs and Scorrano

et al., 2003) provides striking evidence that the communication between the two organelles may depend on the capacity of the cell to transfer Ca^{2+} from ER to the mitochondria. Further along this line, down-regulation of the IP_3Rs has been reported to inhibit apoptosis induced by a variety of mitochondria-linked apoptosis inducers and the cell death response was restored by the expression of IP_3Rs in cells lacking IP_3Rs (Jayaraman and Marks, 1997; Assefa *et al.*, 2004). On the other hand, increased amounts of IP_3R protein were reported in lymphocyte apoptosis (Khan *et al.*, 1996) and in developmental apoptosis during the last stages of embryogenesis (Rosemblit *et al.*, 1999).

A recent study by Boehning and co-workers has shown that cytochrome *c* binds to the IP_3Rs and causes sensitization of the channel activation towards IP_3. Some evidence has also been provided that in cells exposed to staurosporine, cytochrome *c* release causes localized activation of the neighboring IP_3Rs to release Ca^{2+} that is taken up by the mitochondria and is used to amplify the mitochondrial membrane permeabilization in the early stages of apoptosis, before any activation of caspase-3 takes place. This model implies a bi-directional, local signaling mediated by both cytochrome *c* and Ca^{2+} between the mitochondria and the ER (Boehning *et al.*, 2003).

Ca^{2+}-Independent Means of the ER-Mitochondrial Communication

The BH3 only Bcl-2 family protein, Bik is localized to the ER and is able to influence Bax insertion to the mitochondria and cytochrome *c* release from its ER localization. Using an *in vitro* reconstitution system, it was found that the effect of Bik was sensitive to Bcl-2 expression, but was insensitive to bongrekic acid, a PTP inhibitor or to removal of Ca^{2+} by the addition of EGTA (1 mM) or EDTA (5 mM). Furthermore, the Bik effect on the mitochondria required a cytosolic component that is independent of BAX and has not been identified yet (Germain *et al.*, 2002). So far, this study is the only example of an ER-localized Bcl-2s protein influencing the mitochondria integrity in a Ca^{2+} independent way. Furthermore, a recent follow-up study demonstrated that overexpression of Bik also results in Bax/Bak-dependent Ca^{2+} release from the ER, which induces Drp1 recruitment to the mitochondria, followed by mitochondrial fragmentation and cytochrome *c* release (see section "Bcl-2s in the ER" and Germain *et al.*, 2005).

Recently, PACS-2, a novel ER-localized multifunctional sorting protein was shown to control the apposition of the mitochondria with the ER and to promote Bid translocation to the mitochondria and cytochrome *c* release during staurosporine-induced apoptosis. Expression of caspase-resistant Bap31 prevented the PACS-2 depletion-induced mitochondrial fragmentation, demonstrating that the change in mitochondrial shape was dependent on the cleavage of Bap31 to p20 (Simmen *et al.*, 2005). Along these lines, the PACS-2 dependent ER-mitochondrial coupling may be important for the apoptotic signal propagation between the ER and mitochondria.

The apoptotic signal is often propagated from cytosol to the organelles through the translocation pro-apoptotic Bcl-2s to the mitochondria or to the ER (Fig. 3.). An example of a similar mechanism operating between the organelles may be the translocation of c-Abl tyrosine kinase from ER to the mitochondria (see section "Toolbox of the ER for Apoptosis Regulation: Ca^{2+}, Caspase-12, BAP31, JAFRAC2..." and Ito *et al.*, 2001).

Exposure of mitochondria to ROS has been found to stimulate both PTP-dependent and independent mechanisms of the mitochondrial membrane permeabilization (e.g., Madesh and Hajnoczky, 2001). In cells treated with an ER targeted photosensitizer, irradiation induced an impairment of the mitochondria as evidenced by the loss of $\Delta\Psi_m$ and cell death. The cell death was not inhibited by Ca^{2+} chelator or Ru360, an inhibitor of the mitochondrial Ca^{2+} uptake but was sensitive to the singlet oxygen scavenger Trolox. Furthermore, Ru360 suppressed the mitochondrial Ca^{2+} overload but failed to prevent the depolarization (Kessel *et al.*, 2005). This work suggests that during photodynamic stress, a ROS signal can be used by the ER to interact with the mitochondria.

FUTURE DIRECTIONS

One of the most important tasks for the future remains to determine the molecular structure of the PTP and the mitochondrial Ca^{2+} transporters. The progress in this area will help to determine the involvement of the PTP in a variety of disease conditions and to delineate the mechanisms underlying the interactions between the PTP and Bcl-2s in mitochondrial membrane permeabilization. The interaction of the Bcl-2 with the IP_3Rs is increasingly recognized as a major checkpoint in the ER pathway to apoptosis and a similar interaction between OMM channels and Bcl2s is an interesting possibility.

Despite of the emergence of the ER pathway, the ER toolbox to apoptosis is seemingly complex and the mechanisms of the apoptotic transformation of the ER need to be addressed in the future.

Recent studies have proposed that mitochondrial fragmentation is a major step in the recruitment of the

mitochondria to apoptosis. Interestingly, the mitochondrial fragmentation appears to suppress the calcium signal propagation from ER to the mitochondria and interfere with the apoptotic effects of some agents (Szabadkai *et al*., 2004). The relationship between the changes in the organelle morphology, function and ER-mitochondrial communication needs to be explored. A related question is whether the close associations between ER and mitochondria provide an interface where the apoptosis-related proteins of the ER and mitochondrial membranes can directly interact with each other to coordinate the apoptotic activities of the two organelles.

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