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Review

Mitochondrial apoptotic pathways

Nora Mohamad, Alicia Gutiérrez, Mariel Núñez, Claudia Cocca, Gabriela Martín*, Graciela Cricco, Vanina Medina, Elena Rivera, and Rosa Bergoc*.

Radioisotopes Laboratory, School of Pharmacy and Biochemistry, University of Buenos Aires. Buenos Aires, Argentina.
* Members of CONICET

Key words: apoptosis, mitochondria, Bcl-2 family, cytochrome-c.

ABSTRACT: Apoptosis or programmed cell death (PCD) is a physiological process characteristic of pluricellular organisms leading to self-destruction of the cell. It is therefore involved in development, homeostasis and host defense. However, a significant difference has been shown between mammalian cell apoptosis and non-mammalian cell apoptosis: mitochondria are implicated only in the former. Execution of PCD includes the release of several proapoptotic proteins from the intermembrane space of mitochondria. They could exert their actions through a caspase dependent as well as a caspase independent way. On the other hand, regulation of PCD is mainly given by the Bcl-2 family members, which are in turn essentially regulated by activation of death receptors and/or DNA damage. Nowadays, execution of apoptosis is better known than its regulation. Nevertheless, we are still far of a complete understanding of the apoptotic process.

Abbreviations: ADP, Adenosine diphosphate; AIF, Apoptosis-Inducing Factor; Akt, homologues of the v-akt oncogene; ANT, Adenine Nucleotide Translocator; Apaf-1, Apoptosis protease-activating factor-1; ATP, Adenosine triphosphate; Bad, Bak, Bax, Bid, Bim, Members of the Bcl-2 family; Bcl-2, B cell lymphoma-2 protein; BH, Bcl-2 homologue; DIABLO, Direct IAP binding Protein with low pI; DNA, deoxiribonucleic acid; FADD, Fas-associated death domain protein; Fas, receptor of death that belongs to the TNF receptor (TNFR) family; Fas-L, Fas ligand; HSPs, Heat Shock Proteins; HtrA, High-temperature requirement; IAP, Inhibitor of Apoptosis Protein; IMM, Inner Mitochondrial Membrane; IMS, Intermembrane Space; MA, Mitochondrial Anchorage; Noxa, p53-inducible (BH3)-only protein; Omi/$HtrA_2$, mammalian serine protease; OMM, Outer Mitochondrial Membrane; PCD, Programmed Cell Death; PTP, Permeability Transition Pore; Puma, p53- inducible (BH3)-only protein; ROS, Reactive Oxigen Species; SMAC, Second Mitochondrial Activator of Caspase; TNF-a, Tumor Necrosis Factor alpha; TNF-R, Tumor Necrosis Factor Receptor; TRADD, TNF-receptor-1 associated death domain protein; VDAC, Voltage-Dependent Anion Channel.

Introduction

Programmed cell death (PCD) or apoptosis is a physiological process that leads to cellular self-destruction. Apoptosis plays essential roles in development, maintenance of homeostasis and host defense in pluricellular organisms. Dysregulation of this process is implicated in various diseases, ranging from cancer and autoimmune disorders to neurodegenerative diseases and ischemic injuries (Thompson, 1995). A wide range of apoptosis related therapies are currently being developed (Mersich and Gadaleta, 2003).

Cells undergoing apoptosis exhibit a number of characteristic morphological and biochemical changes that allow differentiation from necrotic cells (Thompson *et al*., 1992). Differential features between apoptotic and necrotic cells (Ito and Otsuki, 1998) have been described (Table 1).

Address correspondence to: Prof. Rosa M. Bergoc, PhD. Laboratorio de Radioisótopos,Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. Junín 956 (1113) Buenos Aires, ARGENTINA. FAX: (+54-11) 4964-8202 / 4743-2857. E-mail: rmbergoc@arnet.com.ar / rmbergoc@ffyb.uba.ar
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Genetic studies carried out on the nematode *Caenorhabditis elegans* have permitted the elucidation of the molecular pathways implicated in the regulation of the PCD (Yuan *et al*., 1993; Hengarnter and Horvitz, 1994; Horvitz, 1944; Zou *et al*., 1997; Chang and Yang, 2000). In this pluricellular model, various genes that encode for proteins essential for the regulation and execution of apoptosis were identified. Also, their mammalian homologues were described (Lodish *et al*., 2000) (Table 2).

Essentially, these genes belong to one of three groups:

- ced-9 and Bcl-2 encode for proteins that inhibit apoptosis.
- ced-3 encodes for a protein highly homologous to caspase-9, being both of them responsible for the apoptotic initiation process.
- ced-4 and apoptosis protease-activating factor-1 (Apaf-1) encode for an adaptor protein, which permits the interaction between regulatory proteins, i.e. ced-9 and Bcl-2, and initiator proteins, i.e. ced-3 and caspase-9.

TABLE 1.

Differential features between apoptotic and necrotic cells

Apoptosis	Necrosis
Morphologic Criteria	
Membrane blebbing	Loss of membrane integrity
Cell shrinkage and formation of apoptotic bodies	Cell swelling and lysis
Lack of inflammatory response	Significant inflammatory response
Lysosomal preservation	Lysosomal leakage
Biochemical Criteria	
Induction by physiological stimuli disturbances	Induction by non-physiological disturbances
Energy requirement	Lack of energy requirement
Macromolecular synthesis requirement	Lack of macromolecular synthesis requirement
De novo gene transcription	Lack of *de novo* gene transcription
Nonrandom fragmentation of DNA	Random digestion of DNA

Both morphologic and biochemical characteristics are distinctive between apoptotic and necrotic cells. Adapted from Ito and Otsuki, 1998.

TABLE 2.

Analogy between apoptosis in *C. elegans* and mammals

	regulator	adaptator	effector		
C. elegans	Ced-9 ⊣	Ced-4 →	Ced-3 →	death	
mammals	Bcl-2 ⊣	Apaf-1 →	caspase 9 →	caspase 3 →	death

Both *C. elegans* and mammals present proteins that share their function as inhibitors, adaptators and effectors of PCD. However, there is a great difference between apoptosis in each case: mitochondria are only involved in mammalian cell apoptosis. Adapted from Lodish *et al*., 2000.

Once ced-3 or caspase-9 are activated, they start the execution of apoptosis, through the protease activity on the substrate proteins. Caspases recognize aspartate residues and cleave cystein residues. Such cleavage may in turn activate or inactivate substrate protein functions, inducing typical changes (Table 1) and leading an apoptotic cell to death.

Studies about PCD opened a diversity of ways on trying to understand many diseases. That is why Medicine Nobel Prize 2002 was given to three scientists who contributed to the elucidation of the apoptotic process and its regulation: S. Brenner, J. Sulston and R. Horvitz.

Mitochondria and apoptosis

Since 1990, when B cell lymphoma 2 (Bcl-2) protein was found in the mitochondrial membrane, it has been known that mitochondria were implicated in the regulation of mammalian cell apoptosis (Chen-Levy and Cleary, 1990; Hockenbery *et al*., 1990). And a few years later, several mitochondrial proteins capable of activating cell apoptosis were identified. These proteins normally reside in the intermembrane space of mitochondria and, in response to a variety of apoptotic stimuli, they are released to the cytosol, leading to the execution of apoptosis. These proapoptotic proteins may act either in a caspase dependent form, i.e. cytochrome-c, Omi/HtrA$_2$, and second mitochondrial activator of caspases (SMAC), or in a caspase independent form, i.e. apoptosis-inducing factor (AIF) and endonuclease G. Therefore, mitochondria are implicated in regulation as well as in execution of mammalian apoptosis.

Structure and function of mitochondria

At first, mitochondria were studied as cellular adenosine triphosphate (ATP) producers and, by 1960, the coupling of phosphorylation to electron and hydrogen ion transfer was elucidated (Mitchell, 1961). Thirty years later, the interest in mitochondria rearises, when their role in execution and regulation of PCD was discovered.

The mitochondrion is an organelle encompassed by two membranes, which are dissimilar as in composition, as much as in structure and function (van Gurp *et al*., 2003) (Fig. 1).

The outer mitochondrial membrane (OMM) shows more permeability to molecules of up to 5,000 daltons than the inner mitochondrial membrane (IMM). Such permeability is mediated by a voltage dependent anion channel (VDAC), which represents in its open configuration, the most abundant protein in the outer membrane.

Nevertheless, the OMM is impermeable to the proapoptotic proteins that reside in the intermembrane space (IMS) of mitochondria. Such impermeability is crucial for the regulation and the execution of mammalian apoptosis. The mechanisms by which those apoptosis initiators are released from mitochondria remain controversial and will be discussed below.

Maintenance of the permeability of the OMM, primarily through VDAC, is required to keep the integrity of this membrane and to prevent the release of proapoptotic proteins from the IMS. It is believed that impaired permeability across the outer membrane fa-

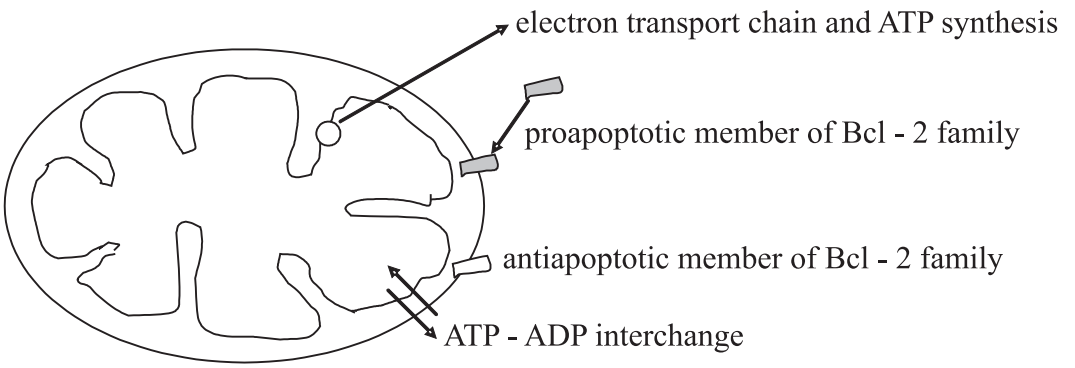

FIGURE 1. Mitochondrial structure.

vors membrane hyperpolarization, increasing reactive oxygen species (ROS) production and matrix swelling.

The inner mitochondrial membrane (IMM) surrounds the mitochondrial matrix and is folded in cristae. The cristae membrane holds the electron transport chain which generates the hydrogen ion gradient necessary to make ATP (Curtis and Barnes, 1989).

When electrons pass throughout the complexes that conform that chain, protons are bombed from the matrix side of the IMM to the IMS side. Since cardiolipin content of the inner membrane makes it highly impermeable to protons, they accumulate in the lumen of the cristae. Finally, the passage of hydrogen ions back to the mitochondrial matrix through the ATP sintetase produces ATP from ADP in the matrix.

Transport of small molecules across the IMM is mediated by carrier proteins and driven by an electrochemical gradient (Lodish *et al*., 2000). Such gradient includes a pH gradient and a voltage gradient. The first one is due to the presence of pH 7 in the intermembrane space side of the inner membrane and pH 8 in the matrix side. The second one is due to the presence of positive charges in the intermembrane space side of the inner membrane and negative charges in the matrix side (Cooper, 1997; Adams and Cory, 1998).

The adenine nucleotide translocator (ANT) in the IMM is juxtaposed in some contact points with the VDAC in the outer, allowing then the import of ADP from the cytosol to the mitochondrial matrix and the opposite export of ATP, driven by the voltage component of the electrochemical gradient. On the other hand, the import of phosphate and pyruvate from the cytosol to the matrix coupled to the opposite export of hydroxyl ions is driven by the pH component of the electrochemical gradient.

The Bcl-2 family

The proteins members of this family regulate apoptosis, promoting or inhibiting the permeabilization and disruption of the outer mitochondrial membrane, after apoptotic process trigger. The Bcl-2 family can be divided into three groups according to its BH domains, its mitochondrial anchorage (MA) and its pro or antiapoptotic action (Budd, 2001; Germain and Shore, 2003) (Fig. 2).

Antiapoptotic members, i.e. Bcl-2 and Bcl-x_L, have BH_1, BH_2, BH_3 and BH_4 domains and a carboxy-terminal hydrophobic transmembrane tail domain, which localizes the proteins to the OMM. Bcl-x_L resides only in the OMM, whereas Bcl-2 also resides in the endoplasmic reticulum and nuclear membranes and translocates to OMM upon an apoptotic signal (Tsujimoto, 1989).

Proapoptotic members include both multidomain and BH_3-only proteins.

- Multidomain proapoptotic members, i.e. Bax and Bak, have BH_1, BH_2 and BH_3 domains and the transmembrane tail domain. It is supposed that Bax and Bak have a cytosolic location and, in response to an apoptotic stimulus, they translocate to OMM. How-

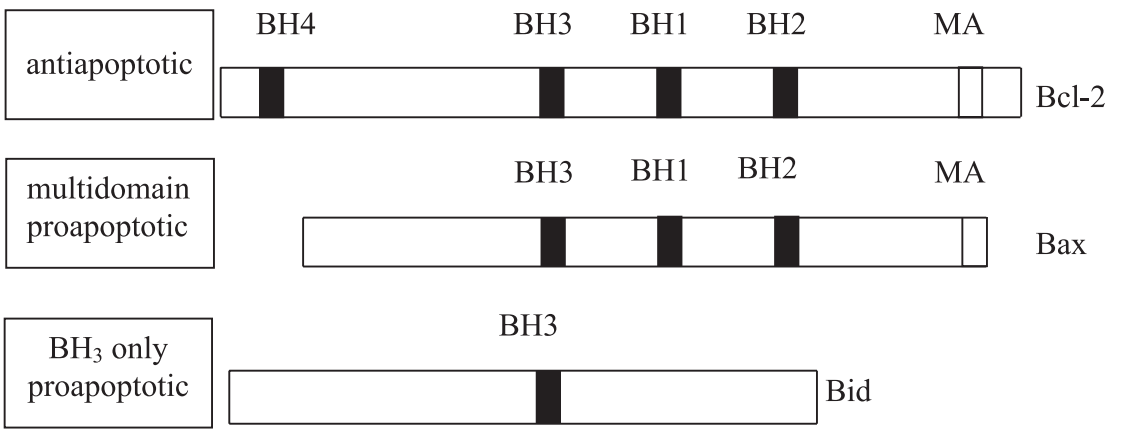

FIGURE 2. Bcl-2 family. The Bcl-2 family could be divided into three groups, according to its BH domains, its mitochondrial anchorage (MA) and its pro or antiapoptotic action. Adapted from Budd R, 2001.

ever, other studies have found that Bak resides in OMM. Anyhow, BH_3-only members interact with multidomain proapoptotic members, inducing apoptosis (Degli Esposti and Dive, 2003; Putcha *et al*., 2002).

- BH_3-only proapoptotic members, i.e. Bid, Bad and Bim, contain only the BH_3 domain and are cytosolic. BH_3 domain is required to interact with other Bcl-2 family members. Following a death signal, BH_3 only proteins undergo post-translational modifications, like dephosphorylation and cleavage. Such modifications result in their activation and translocation to OMM, where they interact with multidomain proapoptotic members, leading to their oligomerization. This might form a pore, big enough for the proapoptotic proteins of the intermembrane space to be released and then apoptosis proceeds (Puthalakath and Strasser, 2002).

Antiapoptotic members neutralize the activity of both multidomain and BH_3-only proapoptotic members. Due to their localization in the OMM, they might associate with multidomain proteins. If Bcl-2 and Bcl-x_L can not exert their antiapoptotic effect, Bax oligomerization is permitted and therefore apoptosis is promoted (Fig. 3).

Regulation of mitochondrial apoptotic signals by BH_3-only proapoptotic proteins

BH_3-only proapoptotic members of Bcl-2 family function as sensors for cellular integrity and functionality: Bid acts as sensor for death domain receptor signaling, Bad does for growth factor withdrawal and Bim acts as sensor for cytoskeleton integrity.

Cleavage of Bid

Activation of cell surface death receptors that belong to the tumor necrosis factor receptor (TNF-R) superfamily in turn, activates caspase-8. It leads to the cleavage of Bid, and then truncated Bid translocates from cytosol to OMM and induces cytochrome c release (Li *et al*., 1998; Luo *et al*., 1998).

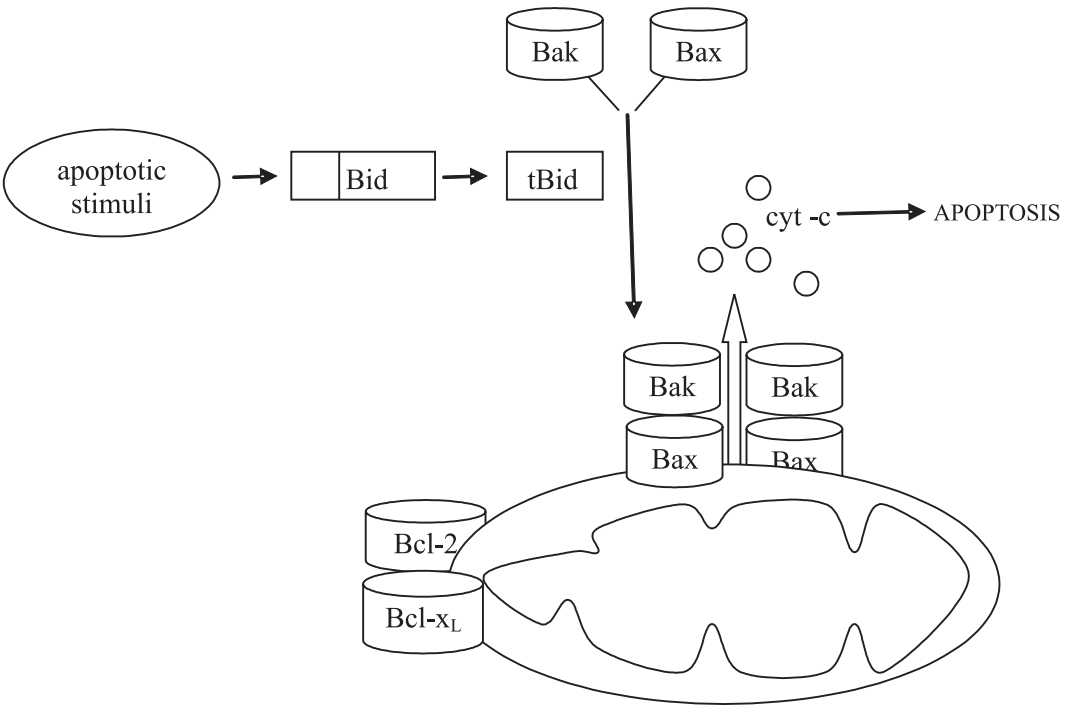

FIGURE 3. Localization and function of Bcl-2 family. Antiapoptotic members reside in the OMM, whereas the proapoptotic members could be in the cytosol and translocate to such mitochondrial membrane. Adapted from Orrenius S, 2004.

Phosphorylation of Bad

In the absence of survival factors, Bad is dephosphorylated. Its BH_3 domain binds and inactivates Bcl-2 and/or Bcl-x_L at the outer mitochondrial membrane, thereby promoting PCD. Conversely, in the presence of survival factors, their specific cell surface receptors are activated, leading in turn to the activation of a series of kinases. Bad is then phosphorylated, allowing it to bind 14-3-3 protein and to remain in the cytosol. In addition, phosphorylated Bad dissociates from Bcl-2 and/or Bcl-x_L, permitting then, survival promotion. There are several phosphatases that dephosphorylate Bad *in vitro*, however, the *in vivo* regulation of these phosphatases remains to be elucidated (Lodish *et al*., 2000; Zha *et al*., 1996).

Dissociation of Bim

Bim has been found associated with microtubule complexes. In response to an apoptotic stimulus, Bim dissociates and translocates to OMM and therefore apoptosis proceeds (Puthalakath *et al*., 1999).

Transcriptional regulation of BH_3-only proteins

This regulation is important when apoptosis requires new protein synthesis, as in DNA-damage induced apoptosis. Transcription of Bax, Noxa and Puma, the two last BH_3-only group members, is induced by p53 (Oda *et al*., 2000; Nakano and Vousden, 2001; Yu *et al*., 2001).

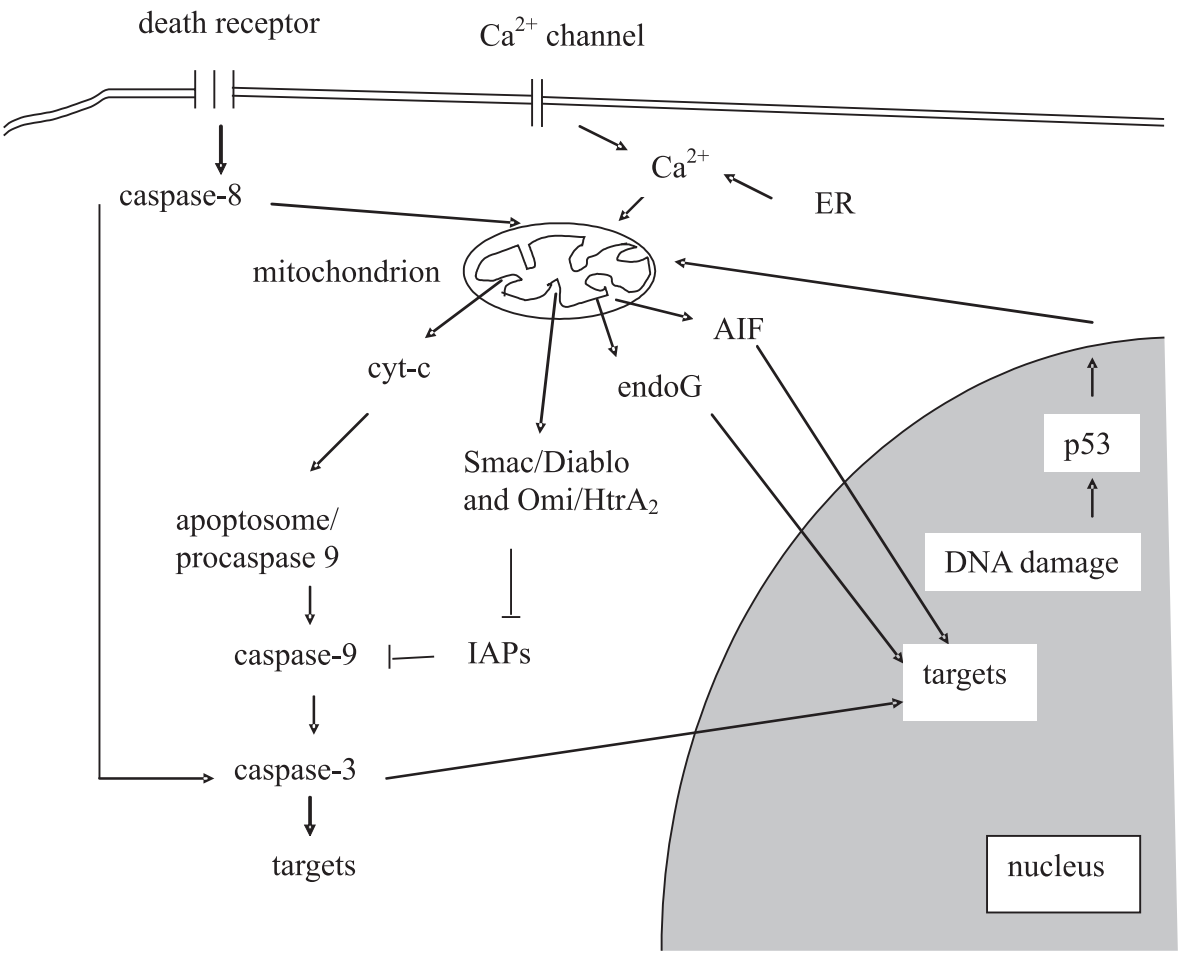

FIGURE 4. Caspase dependent and caspase independent execution of apoptosis. Both death receptor activation, cellular stress and augmented cytosolic calcium can lead to execution of PCD. In turn, this process can proceed in a caspase dependent as well as a caspase independent pathway. The former includes the release of cytochrome-c, SMAC and Omi/$HtrA_2$ from IMS and the direct activation of caspase-3 by caspase-8. The later includes the release of AIF and endonuclease G from IMS.

Caspase-dependent execution of apoptosis

Caspase activation has been described by a receptor-mediated effect, in response to cellular stress, or by an increase in the cytosolic calcium (Wang, 2001) (Fig. 4).

Receptor-mediated caspase activation

In the receptor-mediated caspase activation, the ligand, i.e. Fas-L and TNF-α, binds to its death receptor, i.e. Fas receptor and TNF-R1 (Locksley *et al*., 2001; Chen and Goeddel, 2002; Wajant, 2002). Then, receptor trimerizes and death adapter molecules are recruited on the cytoplasmic side of the membrane. Fas receptor recruits FADD (Fas-associated death domain protein), whereas TNF-R1 recruits TRADD (TNFR1-associated death domain protein) and it in turn recruits FADD. Anyhow, FADD recruits procaspase-8 and it is activated to caspase-8.

The activated caspase-8 can then cleave procaspase-3, yielding caspase-3, which acts as an apoptosis executioner, through the cleavage of multiple substrates whithin the cell. Caspase-8 also activates Bid, which translocates to mitochondria and leads to the release of cytochrome-c, in a way able to be inhibited by Bcl-2. So, cell lines can be classified as type 1, when they can directly produce enough caspase-3 or as type 2, when they depend on mitochondrial amplification.

Caspases activation as a response to cellular stress

On the other hand, caspase activation can be a response to various cellular stresses: DNA damage, protein kinase inhibition and loss of survival signaling. Such stimuli would permit p53-mediated production of Bax, Noxa and Puma, proapoptotic members of the Bcl-2 family (Shen and White, 2001; Vousden and Lu, 2002; Manfredi, 2003). Besides, p53 could induce cell-cycle arrest by allowing p21 transcription. The ability of p53 to produce either apoptosis or cell-cycle arrest depends, partially at least, on its phosphorylation on serine 46. Such phosphorylation is necessary for to induce expression of apoptotic genes, but not cell-cycle arrest mediators genes. Therefore, regulation of serine 46 phosphorylation could be essential in deciding the response to p53 activation.

Calcium-mediated caspase activation

Under apoptotic stimulation, cytosolic calcium increases either by opening plasmatic membrane calcium channels which allow extracellular (EC) calcium to enter or by releasing from intracellular stores, i.e. endoplasmic reticulum (ER). This increase stimulates mitochondrial uptake of calcium, leading to the opening of the permeability transition pore PTP (see below) and so triggering the apoptotic process (Hajnoczky *et al*., 2003; Smaili *et al*., 2003).

Models for the mechanism of cytocrome-c release

The mechanisms by which the outer mitochondrial membrane permeability increases in response to an apoptotic stimulus, as well as the mechanisms by which Bcl-2 proteins regulate this process remain controversial. Two main models have been proposed: the non-specific outer membrane rupture and the formation of specific channels in such membrane. Besides, a third model has been hypothetized as a combination of both. Anyway, all the proapoptotic proteins that reside in the intermembrane space of mitochondria are released into the cytosol, in response to a variety of apoptotic stimuli, promoting the execution of apoptosis.

a. Non-specific outer membrane rupture

Hyperpolarization in response to metabolic changes

In the presence of mitochondrial poisons or under mitochondrial substrate limitations, a reduction in the rate of electron transport can occur (Harris and Thompson, 2000). Thereafter, VDAC closure, hyperpolarization and matrix swelling would lead to OMM disruption and release of proapoptotic proteins to cytosol. However many reports describe such hyperpolarization, other authors describe a loss of transmembrane potential (Quanungo *et al*., 2005). Therefore, another model of non-specific rupture of OMM was proposed.

Permeability transition pore opening

The permeability transition pore (PTP) was proposed to be a multiprotein complex, including VDAC in the OMM, ANT in the IMM and cyclophilin D in the matrix, among others (Crompton, 1999; Tsujimoto, 2003). Multidomain proapoptotic proteins, excessive mitochondrial calcium and ROS induce the opening of the pore, allowing the passage of molecules of up to 1,500 daltons, with loss of IMM integrity and therefore loss of transmembrane potential. Such depolarization is controversial due to multiple reports of mitochondrial

hyperpolarization. Anyhow, matrix swelling, OMM rupture and cytochrome-c release proceed. The PTP formation is inhibited by cyclosporin A, that targets cyclophilin D, by bonkrekic acid, which targets ANT, and by Bcl-2 and Bcl-x_L.

b. Specific-channels formed by multidomain proapoptotic protein oligomerization

Although Bax localization is controversial, it is likely to reside as a monomer in the cytosol, whereas Bak is already an integral protein of the OMM (Degli Esposti and Dive, 2003; Tsujimoto, 2003). Anyhow, Bax or Bak are activated by active BH_3-only proteins. Bax changes its conformation, translocates, inserts into OMM, oligomerizes and can form channels large enough for cytochrome-c to be released. Bak could also oligomerize. VDAC would also contribute to this change of membrane permeability. This channel formation, as PTP formation, can not be inhibited by Bcl-2 nor Bcl-x_L. Multidomain proapoptotic proteins would remain as monomers and VDAC would remain opened as under normal energy metabolism.

c. Complete release of cytochrome-c by two pathways

Mitochondrial cytochrome-c is subcompartmentalized: near 85% resides in the cristae and 15% in the IMS. Despite this, it is fully released, with or without swelling.

It has been proposed (Scorrano and Korsmeyer, 2003) that in response to an active BH_3-only protein, mitochondria initially release only the IMS fraction of cytochrome-c, dependent of its BH_3 domain and of multidomain proapoptotic protein oligomerization.

Thereafter, mitochondria release the cristae fraction of cytochrome-c, but independently of the BH_3 domain and of multidomain proapoptotic proteins oligomerization, indicating cyclophilin D participation in PTP formation. Cristae appear no longer individual, but

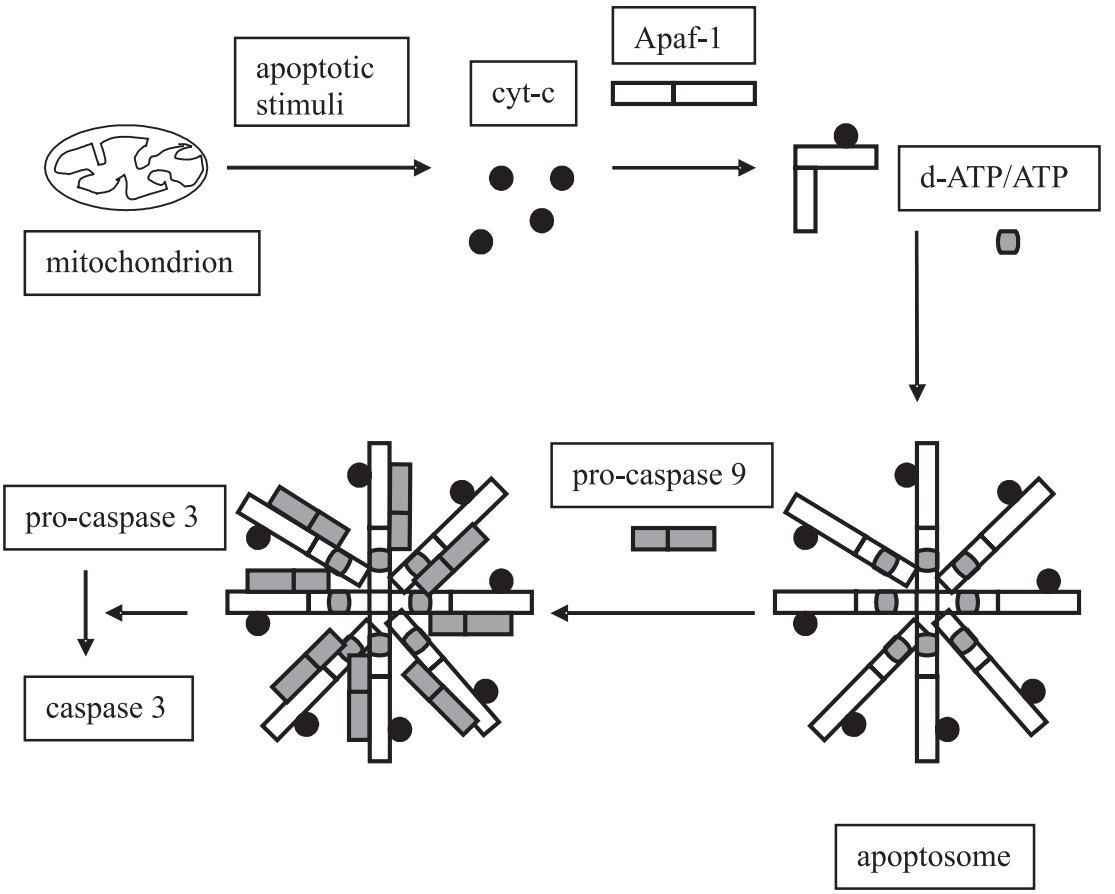

FIGURE 5. Cytochrome-c release. Cytochrome-c release from the IMS leads to the apoptosome formation and, thereafter, to the activation of effector caspases. Adapted from Wang X, 2001.

fused, making cytochrome-c stores completely available to be released across the OMM.

Release of mitochondrial proapoptotic proteins

The execution of PCD through mitochondria is conformed by the release of at least five apoptogenic proteins from the intermembrane space to the cytosol (Hengartner and Horvitz, 1994; Germain and Shore, 2003):

- Cytochrome-c, known since 1996
- AIF, known since1999
- SMAC, known since 2000
- Omi/HtrA$_2$, also known since 2000
- Endonuclease G, known since 2001

Release of cytochrome-c

Once released to cytosol, cytochrome-c binds Apaf-1 (apoptosis protease-activating factor-1), this permits the binding of dATP or ATP and this triggers its oligomerization to form the apoptosome. This allows the recruitment of multiple procaspase-9 molecules to the complex, facilitating their autoactivation. Only so, caspase-9 can cleave and activate executioner caspase-3 and apoptosis can proceed (Li *et al*., 1997; Acehan *et al*., 2002; Adams and Cory, 2002) (Fig. 5).

Release of AIF

AIF (apoptosis-inducing factor) is a 57 kD flavoprotein that is also released to cysosol during apoptosis. As well as SMAC, a precursor is imported to mitochondria. Upon induction of apoptosis, AIF translocates to the nucleus and leads to chromatin condensation and large-scale DNA fragmentation, independiently of caspase activation (Susin *et al*., 1999, 2000; Joza *et al*., 2001; Cande *et al*., 2002).

Release of SMAC

SMAC (second mitochondrial activator of caspases) or DIABLO (direct IAP binding protein with low pI), a 25 kD protein, is also released to cytosol during apoptosis. Its physiological mitochondrial function is still unknown.

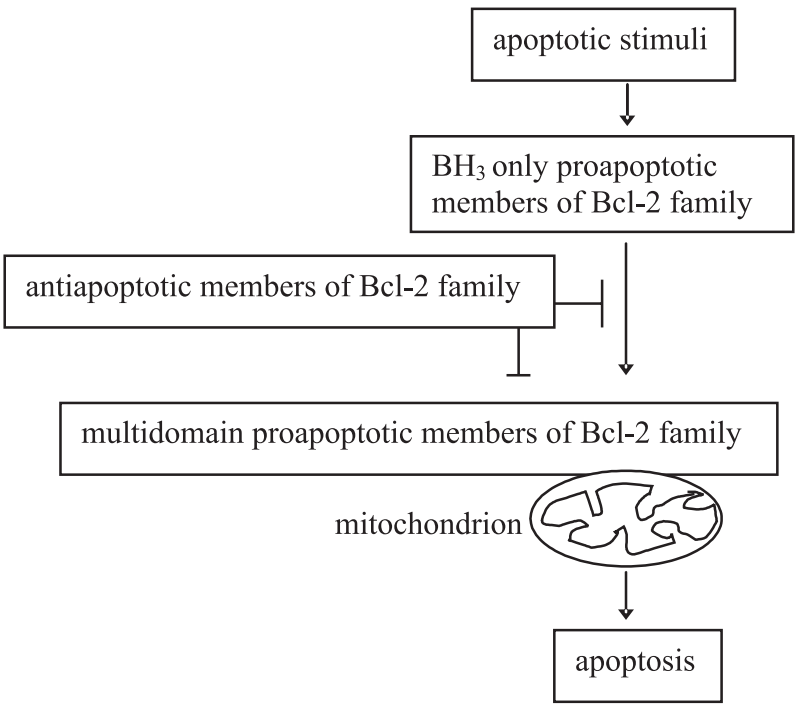

FIGURE 6. Mitochondrial apoptotic pathways today. Upon the regulation of the three groups of Bcl-2 family members, a variety of apoptotic signals can produce the release of the IMS proapoptotic proteins. Once released, they lead to execution of apoptosis.

SMAC precursor presents a N terminus sequence that permits its import to mitochondria. Once in the intermembrane space, that sequence is cleaved, yielding mature SMAC. The new generated N terminus sequence, once SMAC is released, binds to IAPs (inhibitor of apoptosis protein), therefore countering the inhibition of active caspases-3 and 9 by IAPs and ensuring a rapid execution of apoptosis.

It has been suggested that, in a transient release of cytochrome-c, procaspases-3 and 9 would be activated, however, if there is enough IAPs, apoptosis does not proceed. But if mitochondrial damage is severe, SMAC would be released with cytochrome-c, avoiding the caspases inhibition by IAPs and permiting apoptosis to proceed. Besides, it has been proposed that, at first, cytochrome-c is released, this would activate caspases and they would promove release of SMAC, in this way potentiating the apoptotic process (Verhagen *et al*., 2000; Du *et al*., 2000; Wu *et al*., 2000; Srinivasula *et al*., 2001).

Release of Omi/HtrA2

The mammalian serine protease Omi/$HtrA_2$ was identified as a 49 kD protein, homologous to the bacterial endoprotease HtrA (high-temperature requirement). It promotes apoptosis in a similar way to SMAC, that is binding and preventing IAPs action (Gray *et al*., 2000; Faccio *et al*., 2000; Suzuki *et al*., 2001; Verhagen *et al*., 2002; Martins *et al*., 2002; Hedge *et al*., 2002).

Release of endonuclease G

Endonuclease G is a 30 kD protein, encoded by a nuclear gene, translated in the cytosol and then imported to the mitochondria. It has been proposed in 1993 that it would participate in mitochondrial DNA replication that takes place in the matrix. But it was released from the intermembrane space, with the other proapoptotic proteins. Once released, endonuclease G induces DNA fragmentation, independiently of caspase activation, as well as AIF. So, both of them, would trigger an apoptotic pathway parallel to the caspases activation (Li *et al*., 2001; van Loo *et al*., 2001; Widlak *et al*., 2001; Ohsato *et al*., 2002).

Regulation by heat shock proteins (HSPs) of mitochondrial apoptotic signals

Some HSPs are constitutively expressed, whereas expression of the others is induced by a variety of stresses, exerting then a cytoprotective function (Parcellier *et al*., 2003). HSPs facilitate the degradation of proteins by ubiquitin-proteasome system (Mathew *et al*., 2001). Moreover, they are highly expressed in cancer cells, where they participate in oncogenesis, probably by interfering apoptotic pathways (Joattela, 1999).

Mammalian HSPs can be classified in two groups (Garrido *et al*., 2001):

- High molecular weight HSPs, such as HSP90 and HSP70, which are ATP-dependent chaperones and require co-chaperones.
- Small HSPs, such as HSP27, are ATP-independent chaperones and would be modulated by phosphorylation and oligomerization.

Both HSP27 (Garrido *et al*., 1999; Bruey *et al*., 2000; Concannon *et al*., 2001; Paul *et al*., 2002; Pandey *et al*., 2000a) and HSP70 (Creagh *et al*., 2000; Saleh *et al*., 2000; Beere *et al*., 2000; Ravagnan *et al*., 2001) prevent apoptosis by various mechanisms, whereas HSP90 can either facilitate or avoid PCD, according to different apoptotic stimuli (Lewis *et al*., 2000; Pandey *et al*., 2000b; Cardone *et al*., 1998; Sato *et al*., 2000).

Apoptosis and loss of mitochondria functions

Cytochrome-c is the sole water-soluble component of the electron transfer chain. Once released to cytosol, electron flux still occurs, but it is no longer coupled to proton pumping. ATP synthesis is then abolished and electrons escape from the electron transport chain and produce ROS, with subsequent lipid peroxidation. Apoptotic cells show increased cytosolic ADP and decreased cellular ATP content. This would be triggered by the translocation of proapoptotic BH3-only proteins to mitochondria and can be reversed by Bcl-x_L, which promotes survival by avoiding VDAC closure. So, antiapoptotic members of Bcl-2 family would mantain mitochodrial homeostasis (Wang, 2001).

Conclusions

In summary, mitochondrial apoptotic pathways are shown in figure 6. Basically, the release of the proapoptotic proteins from the intermembrane space triggers apoptosis, in a caspase-dependent (through cytochrome-c, Omi/$HtrA_2$ and SMAC) as well as in a caspase-independent form (through AIF and endonuclease G). Besides, activated caspases can cleave antiapoptotic Bcl-2 family members to render proapoptotic proteins, lead-

ing to more mitochondrial damage and, therefore, amplifying the apoptotic signal. Even though caspase-dependent and caspase-independent pathways fail, loss of mitochondrial homeostasis will lead anyhow to cell death. So, apoptosis would be blocked before mitochondrial damage occurs, in order to prevent cellular death. Execution of mitochondrial apoptotic pathway is better known than its regulation. Our knowledge about execution as well as regulation of PCD would have to be much greater to understand why and how a cell decides to live or to die.

Acknowledgments

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