



HAL
open science

Study of PTPC Composition during Apoptosis for Identification of Viral Protein Target

Florence Verrier, Bernard Mignotte, Gwénaél Jan, Catherine Brenner

► **To cite this version:**

Florence Verrier, Bernard Mignotte, Gwénaél Jan, Catherine Brenner. Study of PTPC Composition during Apoptosis for Identification of Viral Protein Target. *Annals of the New York Academy of Sciences*, 2003. hal-03034595

HAL Id: hal-03034595

<https://hal.science/hal-03034595>

Submitted on 1 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

STUDY OF PTPC COMPOSITION DURING APOPTOSIS FOR IDENTIFICATION OF VIRAL PROTEIN TARGET

Florence Verrier¹, Bernard Mignotte¹, Gwenaël Jan² and Catherine Brenner¹

¹ CNRS FRE 2445, Université de Versailles/St Quentin, 45, avenue des Etats-Unis, 78035 Versailles, France;

² Institut National de la Recherche Agronomique, UR 121, Laboratoire de Recherches de Technologie Laitière, 35042 Rennes Cedex, France;

Running title: Dynamic of PTPC composition

Correspondence: **CNRS FRE 2445,
Université de Versailles / St Quentin,
45, avenue des Etats-Unis,
78035 Versailles, France
Tel: 33 1 39 25 45 52
Fax: 33 1 39 25 45 72
E-mail: cbrenner@genetique.uvsq.fr**

Abbreviations: ANT, adenine nucleotide translocator; CypD, Cyclophilin D; $\Delta\Psi_m$, mitochondrial transmembrane potential; MMP, mitochondrial membrane permeabilization; Vpr, vMIA, HBx, M11L; IM, inner membrane; OM, outer membrane; PTPC, permeability transition pore complex; PBR, peripheral benzodiazepin receptor; SDS-PAGE; ROS.

Abstract

The permeability transition pore complex (PTPC), a mitochondrial polyprotein complex, has been previously described to be involved in the control of the mitochondrial membrane permeabilization (MMP), during chemotherapy-induced apoptosis. PTPC may contain proteins from both mitochondrial membranes (e.g. voltage dependent anion channel (VDAC), PRAX-1, peripheral benzodiazepin receptor (PBR), adenine nucleotide translocator (ANT)), from cytosol (e.g. hexokinase II, glycerol kinase), from matrix (e.g. cyclophilin D (CypD)), and from intermembrane space (e.g. creatine kinase). PTPC may also interact with tumor suppressor proteins (i.e. Bax, Bid), oncoprotein homologs of Bcl-2 and some viral proteins, which can regulate apoptosis-induced by pore opening. ANT and VDAC are the target of numerous pro-apoptotic MMP inducers. However, the precise composition of PTPC as well as the respective role of each PTPC component represents a major issue in the understanding MMP process. Using several experimental strategies, as a combination of co-immunoprecipitation, proteomics and also functional tests with proteoliposome, we and other have been able to characterize some of the intra/inter-PTPC protein interactions leading to a better understanding of the process of MMP. In addition, this approach could identify new putative members and regulators of PTPC pro-apoptotic function and serve to find new targets of viral protein involved in the modulation of apoptosis during infection.

Mitochondria are well known as sites of electron transport and generators of cellular ATP, but have also been described as one site of the regulation of cell survival. Mitochondrial membrane permeabilization (MMP) is a key event leading to physiological or therapy-induced apoptosis (1, 2). Some evidences propose that this event can be caused by the opening of the mitochondrial megachannel, also called the permeability transition pore complex (PTPC) and is controlled by Bcl-2 family member's (3, 4) and by a variety of apoptosis-inducing stimuli such as pro-oxidant agents (5, 6), adenine nucleotide translocator ligands (5), viral proteins (7-9), bacterial metabolites (propionate, acetate, (10)), as well as chemotherapeutic agents (11, 12). This review will focus on characterization of PTPC members as well as modulator proteins that can physically and functionally interact with these proteins during apoptosis.

1. Methodology

In order to identify new proteins members associated with and/or regulating the PTPC susceptible to regulate the mitochondrial phase of apoptosis and also to be the target of viral proteins, we used the methodology described in Figure 1. Mitochondria were first purified from healthy rat organs (heart, liver and brain) or from human tumoral cell lines and solubilized with Triton X-100 detergent. Proteins interacting with PTPC were isolated by immunoprecipitation using a polyclonal rabbit serum specific to adenine nucleotide translocator (ANT), the major mitochondrial inner membrane (IM) protein. Immuno-complexes were then characterized by one-dimensional-electrophoresis (1D-SDS-PAGE) and western-blot (13) or by two-dimensional-electrophoresis (2D-SDS-PAGE) followed by proteomic (14). To study the functional role of these proteins in the regulation of the pore opening, two tests have been used. PTPC, purified from rat brain mitochondria (15, 16) or ANT, purified from rat heart mitochondria (17, 18) were reconstituted in liposomes or planar lipid bilayers (4) (Figure 1), and have been shown to exhibit a functional behavior comparable with that of the PTPC present in intact mitochondria. In these models, PTPC or ANT form non-specific pores. In the ADP/ATP translocase assay, translocation function of ANT was measured by the release of ATP preloaded in ANT-liposome induced by exogenous addition of ADP. Quantification of ATP released was measured by emitted luminescence after addition of luciferase (11). In the pore-opening assay, 4-methylumbelliferyl phosphate (MUP) was encapsulated into ANT-liposome or PTPC-liposome. The conversion into a non-specific pore was measured by the release of MUP which reacts with alkaline

phosphatase and is converted into fluorochrome 4-methylumbelliferone (MU) (5). The electrophysiological assay, as previously described (4, 19), was used to investigate pore-forming activities of ANT, Bax, Bcl-2 and mutants of Bax and Bcl-2, at both macroscopic (black membrane) and single-channel (patch clamp) levels (4). By this technique it has been shown that ANT, after addition of atractyloside (Atr) or Ca^{2+} , mediated a significant conductance in planar lipid bilayers and that the channel formed by ANT is cation specific (4). Furthermore, Bax and Bcl-2 capacity to interact with ANT and to form new channels has been characterized as described later in this review. Using the proteoliposome method, PTPC has been shown to be the target for apoptosis regulation by caspases and Bcl-2 related proteins. Although Ca^{2+} , pro-oxidants, and several recombinant caspases enhance the permeability of PTPC-containing liposomes, and recombinant Bcl-2 or Bcl-XL increase the resistance of the reconstituted PTPC to pore opening (20). Moreover, the same methodology lead us to demonstrate that ANT possesses two opposite functions, ADP/ATP translocator in physiological conditions and a lethal pore in apoptosis (13). This assay has also been used to identify molecules modulating the pro-apoptotic function of ANT, such as viral proteins and chemotherapeutic agents (for review, (21)). Thus, atractyloside, Ca^{2+} , Bax, thiol cross-linkers (diazenedicarboxylic acid bis 5N, N-dimethylamide, dithiodipyridine, bis-maleimido-hexane, tert-butylhydroperoxide), nitric oxide (5, 6), Vpr from HIV-1 (7, 8), short-chain fatty acids (10), or chemotherapeutic agents such as lonidamine, arsenic trioxide, CD437, or verteporfin can convert ANT into a non-specific pore (11, 12). In contrast bongkreikic acid (BA), Bcl-2, vMIA, a cytomegalovirus-encoded protein (9), peptide ANT104-116, ATP and ADP inhibit the pore formation. Finally, using proteoliposome, we showed that Bax, co-reconstituted with ANT increase the Atr-dependent pore opening since Bcl-2 decrease this effect (3). In contrast Bax inhibits translocase activity of ANT since Bcl-2 facilitate ATP/ADP translocation (13).

Viral proteins have been described to regulate apoptosis at a mitochondrial level, as described later in this review. The methodology described above (proteoliposome) could be used to characterize functional interaction between viral protein and mitochondrial proteins, as it has been previously described with Vpr and vMIA. Indeed, identification of new mitochondrial protein targets for viral proteins, could be achieved by co-immunoprecipitation, in infected cells or in cells overexpressing viral proteins. Furthermore,

knowing the mitochondrial target, cytoprotector peptides derived from the target sequence, could be used as inhibitor of viral/mitochondrial proteins interaction and to prevent virus-induced apoptosis.

2. PTPC composition evolution

Classical members of PTPC

The PTPC is a polyprotein complex formed in the contact site between the inner (IM) and the outer mitochondrial membranes (OM) that participate in the mitochondrial matrix homeostasis and the regulation of MMP, a process leading either to apoptosis, necrosis or autophagy (21). In physiological conditions, PTPC controls mitochondrial Ca^{2+} homeostasis via the regulation of its conductance, by the mitochondrial pH, the inner transmembrane potential $\Delta\Psi_m$, NAD/NAD(P)H redox equilibrium and matrix protein thiol oxidation (22-28). As measured by electrophysiology, PTPC exhibits multiple conductance states, suggesting that the channel is composed of several cooperating subunits (29). Its opening as a large unselective channel results in an increase in the permeability of the IM, permitting the influx or efflux of any molecule of $\text{MM} \leq 1500$ Da. By contrast, in lethal conditions, the extended opening of PTPC would lead to an irreversible MMP, a rupture of the OM and the release of apoptogenic proteins in the cytosol, that triggers the ultimate degradation phase of apoptosis and the cell death (2, 21, 30). Depending on the conditions of apoptosis induction, PTPC opening has been shown to precede Bax translocation from cytosol to mitochondria (31) or to occur concomitantly with Bax translocation, but before cytochrome *c* or apoptosis inducing factor (AIF) release (for review: (2)). Thus, because of its capacity to control MMP, alterations of PTPC function may lead to excess or lack of apoptosis and to numerous pathologies, such as ischemia-reperfusion (32), cardiomyopathy (33), neurodegeneration (34), lupus erythematosus (35) or Cancer. The precise composition of PTPC has been a matter of debate. Based on, at least, co-purification experiments, several non-mutually exclusive models have been proposed. The first model described the PTPC as composed by three proteins, ANT (the major protein of the IM), VDAC (the most abundant protein of the OM), both enriched at mitochondrial contact site, and the peripheral benzodiazepin receptor (PBR, in the OM) (36), being implicating this protein complex in lipid metabolism by facilitating the transfer of cholesterol to the IM in steroidogenic cells (37). Others have described PTPC as composed of ANT, VDAC and cyclophilin D (CypD, in the matrix) (38) or as a channel unit formed by ANT and

VDAC, this channel being regulated by CypD (39). Finally, a model has been proposed in which PTPC contains, at least, hexokinase (HK, in the cytosol), PBR, VDAC, ANT, creatine kinase (CK, in the intermembrane space) and CypD (in the matrix) (15, 20, 21, 40) (Figure 2). The association of proteins to ANT/VDAC complexes may be related to cellular energy metabolism. Indeed, creatine kinase (mitochondrial intermembrane space) has been shown to be associated with ANT/VDAC complex (41). Thus ATP can translocate via ANT to generate creatine phosphate, which is then exported via VDAC to the cytosol where it is highly diffusible. In the search for the precise composition of PTPC, we found, by coimmunoprecipitation of solubilized mitochondrial membrane with an anti-ANT serum, followed by 1D-SDS-PAGE and western-blot analysis, that ANT associated with VDAC, HK, Bcl-2, PBR, CypD, in mitochondria isolated from various normal tissues (rat brain, heart and liver) and three human cancer cell lines (HT29, HeLa, and MCF-7) (14). This observation is consistent with the last model previously described above. Furthermore by 2D-SDS-PAGE analysis, we showed that almost 10 major proteins are co-immunoprecipitated with ANT and that some interactions seem to be favored during apoptosis induction while others seem to be disrupted (14).

Moreover, we and other groups have demonstrated, that PTPC function could be modulated by physical interaction of some of its members with onco- and anti-oncoproteins from the Bax/Bcl-2 family. Thus, ANT and VDAC were identified as ligands of Bax, Bcl-2, Bid, Bcl-XL (18, 23, 42, 43), as regulators of channel activity (4, 44), and as modulators of the ADP/ATP translocase activity of ANT (13).

Bax/Bcl-2 family members

Bcl-2 family members have been described to regulate and control the mitochondrial phase of apoptosis (for review, (45, 46). They can be grouped into three categories, the anti-apoptotic members including Bcl-2, Bcl-xL, Bcl-w, Mcl-1, the multi-domain pro-apoptotic members, such as Bax and Bak, and the BH3 domain only proteins including, Bim, Bid, Bad and Bik (47). Generally, proapoptotic members are localized in the cytosol and move to mitochondria upon induction of apoptosis, whereas anti-apoptotic members are localized in intracellular membranes, being predominantly enriched in the contact sites of mitochondrial membranes where the PTPC is also localized (for review, (48). Different roles have been conferred to Bcl-2 like proteins: autonomous channel formation, mitochondrial channel regulation,

increase of cell resistance to pro-oxidant stress or interaction with members of the apoptosome (49-54), however their exact apoptosis mode of action is still elusive. Various different experimental models have been used, such as artificial membrane, isolated mitochondrial membrane, extracted mitochondrial proteins or recombinant protein and several hypothetical mechanisms have been proposed to explain their regulatory actions on apoptosis. Two major mechanisms for permeabilization of the OM have been suggested and summarized in Figure 3: (i) the formation of specific channels into the OM (Bax, Bak, Bcl-xL and Bid forms by itself channels, Bax destabilized the mitochondrial membrane inducing lipidic holes (55), Bax forms chimerics channels with VDAC or ANT) and (ii) opening of the PTPC leading the mitochondrial matrix swelling and rupture of the OM.

Under physiological cellular conditions, Bax has been described to be monomeric and cytosolic (56, 57). During apoptosis, Bax changes its conformation, maybe by change in cytosolic pH (56), by direct interaction with Bid (58, 59) or as the result of changes in mitochondrial membrane potential (31), translocates to the mitochondria and oligomerizes, causing cytochrome *c* release from mitochondria (58, 60, 61). Multimerization has been shown to be sufficient for Bax translocation (57) and oligomeric Bax is able to induce cytochrome *c* release from isolated mitochondria (60). It has been suggested that anti-apoptotic Bcl-2 family members could inhibit Bax conformational change by direct interaction (62). Moreover, anti-apoptotic Bcl-2 members can inhibit cytochrome *c* release from mitochondria by preventing translocation and/or activation of Bax on the mitochondria (63, 64). Bcl-2 and Bcl-xL can also inhibit apoptosis in Bax-independent manner (65, 66). On the other hand, if BH3 proteins can activate pro-apoptotic Bcl-2 members, they have been described as inducing apoptosis by binding anti-apoptotic Bcl-2 members and inhibit their activity (67, 68).

One mechanism for the release of cytochrome *c* and other pro-apoptotic proteins from mitochondria intermembrane space is the formation of specific channels in the OM. Some Bcl-2 family members possess structural similarities with to pore-forming bacterial toxins (69-71), and Bcl-xL, Bid, Bcl-2 and Bax have been shown to form protein artificial membranes (50, 72). However, it has been shown that Bid, on its own, has no effect on cytochrome *c* release in vivo (58). Several experimental evidence supports the idea than Bax or Bak can form ion channels in OM and trigger cytochrome *c* release. Indeed, Bax and Bak could form oligomers and tetramers of Bax could form a pore large enough to release cytochrome *c*

and dextrans (73). In addition, recombinant Bax is able to form channels in artificial membrane without any additional proteins (49) which would be able to permeabilize lipids membranes to cytochrome *c* (74). Thus, it has been shown that Bax forms large oligomers in the OM of cells undergoing apoptosis (63, 75, 76). However, it remains unclear that these oligomers are exclusively composed of Bax or contains others proteins. Indeed, Bak was found to be associated with these large clusters of Bax (76). Several experimental evidences also support the idea that Bax or Bak can form ion channel in OM and trigger cytochrome *c* release without mitochondrial swelling (77, 78). Furthermore, in an other study, cytochrome *c* depletion of isolated mitochondria by addition of Bax or Bid was not accompanied of the loss of mitochondrial transmembrane potential, suggesting that the IM remained intact (79). Bid-induced cytochrome *c* release is Bak dependent in mouse liver mitochondria, and is not inhibited by PTP inhibitor cyclosporin A (58). Finally, a recent study shows that in a cell free system, Bid (its BH3 domain) activates monomeric Bax to produce supramolecular membrane opening, allowing the passage of very large proteins (2 mega Dalton). This process is inhibited by Bcl-XL (80).

The intracellular redox state has been shown to play an important role in many types of apoptosis induced by TNF, UV, growth factor withdrawal and ceramide (81, 82). Bcl-2 might act as an antioxidant partner to block a putative reactive oxygen species (ROS)-mediated step in the cascade of event required for apoptosis. Thus in some studies Bcl-2 seems to stimulate the generation of ROS (83, 84) whereas in others Bcl-2 will prevent oxidative damage (85-87). However, the way by which Bcl-2 protects cell from ROS is unclear and so far no direct evidence has been shown to prove this function.

The involvement of VDAC in Bax-induced apoptosis has also been supported. Using immunoprecipitation, it has been shown that Bax interacts with VDAC during apoptosis (23) and electrophysiological experiments have demonstrated that Bax and VDAC reconstituted in liposomes form a channel larger than either VDAC or Bax alone (44). Furthermore, Bax and Bak enhanced the permeability of VDAC-liposome to cytochrome *c*. In another study antibodies against VDAC were shown to prevent Bax-induced apoptosis while having no effect on Bid- or Bik-triggered apoptosis. Furthermore, it has been reported that VDAC was necessary to the binding of Bax and Bak to red blood cells (88). On the other hand, it has been reported that O_2^{\bullet} -induced cytochrome *c* release could be mediated by a pore containing

VDAC (89). Furthermore, a recent study shows that Bim can interact and activate directly VDAC and that this interaction is enhanced during apoptosis (90).

Interactions between Bax and ANT have also been demonstrated by co-immunoprecipitation. Additionally, Bax was shown to induce cell death when expressed in wild type yeast but not in an ANT-deficient yeast strain (3). In response to Atr or Ca^{2+} , ANT can form channels in lipid membranes (17, 91). Bax has been shown to enhance the ANT channel activity while endogenous ligand ATP inhibits this activity, suggesting that conformational change in ANT is necessary to this channel (4). In contrast, ANT and Bcl-2 do not form channel, Bcl-2 prevents formation of Atr-treated-ANT channel and inhibits the cooperative Bax/ANT interaction to form a channel. It has further been demonstrated that ANT/Bax channel was cationic as Bax channel was anionic, suggesting that Bax and ANT form a cooperative channel. In a recent study it has been shown that Bcl-2 enhances the ATP/ADP exchange in proteoliposomes containing ANT, in isolated mitochondria and mitoplasts, as well as in intact cells, while Bax, which displaces Bcl-2 from ANT in apoptotic cells, inhibits ATP/ADP exchange through a direct action on ANT. Bax-mediated inhibition of ATP/ADP exchange can be separated from Bax-stimulated formation of non-specific pores by ANT, indicating a double functional interaction with ANT (13). In agreement with the model of the PTPC opening regulation by Bax via an interaction with ANT, we have shown a dynamic interaction between PTPC and Bax and Bcl-2 during apoptosis. Thus, in mitochondria of cells treated with etoposide, Bax association with PTPC (proteins co-immunoprecipitated with ANT) displays a dramatic increase since the Bcl-2/PTPC interaction decreases (13, 14), concomitantly with the loss of $\Delta\Psi_m$ (Figure 4). Furthermore, while the interaction of Bcl-2 family members with PTPC changes early after the treatment, other interactions between ANT, VDAC and hexokinase were maintained until chromatin condensation and nuclear degradation occurred, suggesting that they are critical for apoptosis (14).

3. Viral proteins targeting PTPC components and regulating apoptosis.

Viruses are known to negatively or positively modulate apoptotic response (92). Viral proteins target to mitochondria have provided a molecular link between virus infection and induction or suppression of apoptosis. A large number of viral apoptotic modulators that influence mitochondrial cell death

pathways are being identified and are summarized in Table 1. Some of these viral proteins have been described to target mitochondria, and others bind directly to PTPC component.

Bcl-2 homologs

The genome of several viruses code for death-inhibitory Bcl-2 analogs which localize to mitochondria and may interact with pro-apoptotic Bax homologs. Some proteins present sequence homology with Bcl-2 and Bcl-XL, yet their role in regulation of apoptosis has not been demonstrated so far. Viral anti-apoptotic Bcl-2 homologs include LMW5-HL of African Swine fever Virus (93, 94) and the murine gamma Herpes Virus protein, M11 (95), the Bcl-2 homolog of Bovine Herpes Virus 4 (96), BALF-1 of Epstein-Barr virus (97) and BHRF-1 of Herpes Virus Papio (98). In addition, KsBcl-2 of Human Herpes Virus 8 has been shown to be anti-apoptotic and localize to cytoplasmic membrane structure (99). Other viral Bcl-2 homologs have also been described to display a direct, proximal effect on mitochondrial function when expressed in cells independently of other viral proteins. Thus the BHRF-1 protein of Epstein-Barr virus displays both anti-apoptotic function (100) and mitochondrial localization (101). This viral protein, as well as E1B19K protein of adenovirus, are both able to abolish apoptosis induced by ROS, which disrupt mitochondrial integrity (102). Furthermore, E1B19K protein can interact with Bax, providing a protective effect against Bax-induced mitochondrial $\Delta\Psi_m$ loss and apoptosis (103). Furthermore, E1B19K inhibit Bax oligomerization and block TNF- α -induced cell death (104). HVS-Bcl-2 of Herpes virus saimiri is also anti-apoptotic (105) protecting cells from apoptosis induced by Fas ligation, dexamethazone treatment, DNA damage or O_2^{\bullet} production, and on the mitochondria preventing of $\Delta\Psi_m$ loss, cytochrome c translocation into cytosol and caspase-3 activation (106).

Mitochondrial modulatory proteins

Some viral proteins have been described as pro-apoptotic. p13II protein of Human T cell leukemia lymphotropic virus type 1 has been reported to accumulate in the IM and induce an alteration in mitochondrial morphology as well as $\Delta\Psi_m$ loss (107, 108). Nevertheless, so far, increased levels of apoptosis in p13II-expressing cells have not been demonstrated (107). Furthermore, the mitochondrial effects of the synthetic peptide spanning residues 9-41 of p13II including the amphipatic mitochondrial targeting sequence, were insensitive to cyclosporin A, suggesting a permeability transitory pore-independent mechanism (108). Apoptin, of chicken anemia virus induces cell death in a caspase dependent-

manner. However in a presence of caspase inhibitors, this protein is able to induce $\Delta\Psi_m$ loss and cytochrome *c* release (109). The E6 protein of human papillomavirus type 16 sensitizes cells to apoptosis induced by Atr. This effect is p53 and caspase dependent and is abolished by cyclosporin A (110). The transactivating protein Tat of Human Immunodeficiency virus type I (HIV-1), has been implicated in triggering apoptosis by a variety of mechanisms including the mitochondrial cell death pathway (111). Stable expression of Tat in hippocampal neurons induces caspase-dependent cell death associated with an increase in mitochondrial Ca^{2+} level and an accumulation of ROS (112). Involvement of the mitochondrial membrane permeability transition is suggested by the finding that apoptosis is prevented by cyclosporin A.

Direct interaction between viral proteins and PTPC.

The importance of the PT pore in the context of apoptosis modulation in infected cells has been emphasized by the identification of several viral proteins that physically associate with PTPC members (Figure 5). Some viral proteins exert proapoptotic effect (Vpr and Tat of HIV-1, and HBx of HBV) while others inhibit apoptosis (*v*-MIA of CMV, M11L of Myxoma Virus and BHRF1 of EBV).

Vpr of HIV

Vpr, a small accessory protein encoded by HIV-1, has been shown to induce MMP via a specific interaction with PTPC (7). A synthetic peptide, corresponding to the C-terminal moiety of Vpr (aa 52-96), uncouples the respiratory chain and induces a rapid inner MMP to protons and NADH. In isolated mitochondria, this peptide induces matrix swelling and inner MMP, both prevented by pre-incubation with Bcl-2 protein (8). Furthermore it has been shown that Vpr52-96 can interact with ANT and that purified ANT in association with Vpr form large conductance channels in artificial membrane (8). This cooperative channel formation relies on a direct protein-protein interaction since it is abolished by the addition of a peptide corresponding to the Vpr binding site of ANT (104-116), the same domain that interacts with Bcl-2 (3). Bax and Bcl-2 have been shown to modulate the functional and physical Vpr/ANT interaction. Indeed, while Bcl-2 inhibit this interaction, Bax increases the conductance of Vpr/ANT channel (8). Furthermore, the interaction of Vpr and ANT interaction involves an α -helical dodecapeptide domain (aa71-82) of Vpr (113), characterized by the presence of three critical arginine residues, crucial for 71-82 peptide to induce MMP on purified mitochondria (8, 114).

vMIA of CMV

Human cytomegalovirus (CMV) is a Herpes virus that causes opportunistic infections in immunocompromised individuals. CMV inhibits apoptosis mediated by death receptors and encodes a viral mitochondria-localized inhibitor of apoptosis, namely vMIA. This viral protein is capable of suppressing apoptosis induced by diverse stimuli, as ligation of the death receptors Fas/Apo-1/CD95, TNF receptor I, TRAIL, staurosporine and maytansine, and pro-oxidants (6, 115). vMIA is predominantly localized in mitochondria, where it has been shown to be associated with ANT. These functional properties resemble those ascribed to Bcl-2. However, the absence of sequence similarity to Bcl-2 or any other known cell death suppressors suggests that vMIA defines a new class of anti-apoptotic proteins preventing cell death by a direct interaction with ANT. Unlike Bcl-2 family members (23), vMIA does not bind VDAC (6, 115). Also, in contrast to Bcl-2, vMIA does not bind Bax (9). Mutagenesis studies have shown that two regions of vMIA are essential for its anti-apoptotic action. The N terminal domain (amino acids 5-34) is required for mitochondrial localization, and the second domain 118-147, conserved among different cytomegalovirus isolates, of which mechanistic function is not yet known. vMIA inhibits Fas-mediated apoptosis at a point downstream of caspase-8 activation and Bid cleavage but upstream of cytochrome *c* release. Mitochondria from cells overexpressing vMIA are resistant to cytochrome *c* release induced by truncated Bid (tBid) (116). It has been proposed that vMIA could interfere with tBid inhibiting the permeabilization of the OM induced by the latter (116).

HBx of HBV

Hepatitis B virus X protein (HBx) is implicated in the development of hepatitis B virus-induced hepatocellular carcinoma. HBx possesses multiple functions that can lead to either cellular proliferation or to apoptosis (transcriptional activation, disruption of p53 activity, DNA repair, stimulation of kinase signaling pathway activating mitotic Raf, Ras and MAP cellular (117-121). HBx is a potent apoptosis inducer, whose effects are neutralized by Bcl-2 or not (122, 123). The C-terminal region of HBx is involved in the inactivation of p53 inhibiting apoptosis (124, 125). On the other hand, HBx possesses two domains that inhibit kinase/serine (126) and caspases (127). Thus expression of HBx in rat fibroblast and hepatoma cells induces cellular resistance at several apoptotic stimuli (Fas antibodies and growth factor

withdrawal) which engage caspase 3 (127). Hbx can also induce spontaneous apoptosis in primary hepatocyte and in liver of HBx transgenic mice (128-130). Furthermore, HBx can render human hepatocyte cells and rat fibroblasts sensitive to the apoptosis induced by TNF- α (131, 132). Interestingly, HBx has been localized to mitochondria and shown to promote the $\Delta\Psi_m$ loss when expressed independently of other viral proteins (133, 134). A more recent study has shown that the transmembrane region (aa54-70) is required in the mitochondrial targeting of HBx and that the putative α -helical regions (aa75-88 and aa 109-131) is involved in this targeting (135). In addition to its mitochondrial localization, HBx interacts with VDAC (134). These protein sequences possess significant homology (HVDAC3 and HBx possess 31% amino acids similarity). Furthermore, a C-terminal HBx deletion mutant, HBx Δ 99, failed to bind VDAC and activate STAT-3 and NF- κ B (135). As described above, HBx possesses both pro- or anti-apoptotic activity. It has been proposed that the pro-apoptotic activity facilitates viral propagation and lead the immune system to escape (136). Controversial effects on HBx on apoptosis could be due to different modes of action according to HBx concentration at several stages of natural HBV infection. That is, HBx could inhibit apoptosis at early stage of infection while in a later stage of infection, it could induce apoptosis favoring HBV replication and propagation. Pro and anti-apoptotic effect could also result in the malign transformation of hepatocytes. Apoptosis inhibition could then permit transforming mutations. Stimulation of hepatocyte proliferation in response to pro-apoptotic effect could lead to the selection of pre-maligned cells in the liver. This effect could increase gene activation and regulation of cellular growth (137).

M11L of Myxoma virus

The myxomavirus protein, M11L prevents apoptosis during virus infection (138). In vitro, M11L knockout viruses cause accelerated apoptosis in infected rabbit lymphocytes or monocytes (138, 139). M11L has also been reported to target the mitochondrial and inhibit $\Delta\Psi_m$ loss and apoptotic changes (caspase-3 activation, DNA fragmentation), induced by staurosporine (138). A recent study has shown that M11L is physically associated with the mitochondrial PBR (component of the PTPC) and, expressed in stable cell line, inhibits the release of cytochrome *c* and $\Delta\Psi_m$ loss induced by staurosporine, alone or in combination with pro-apoptotic ligands of PBR. This indicates that M11L functionally prevents transmission of apoptotic signals that proceed via mitochondria (140). This represents the first evidence

that the PBR may physically associate with an anti-apoptotic protein able to modulate mitochondrial function. Interestingly, expression of the non-targeted M11L truncation mutant protein in stable cell line, failed to protect it from apoptosis. Furthermore, it has been described in the same study that M11L/PBR functional interaction was not disturbed by the binding of PBR ligand, FGIN-1-27, suggesting that M11L acts more as a modulator of PBR than as a competitive molecule (140, 141). The mechanism by which M11L could inhibit apoptosis is not fully understood, but some hypotheses have been proposed. PBR is an OM protein, possessing five transmembrane structures and is involved in several functions including cholesterol transport from the OM to the IM, regulation of steroidogenesis, porphyrin transport, sensing of ROS species and regulation of apoptosis (142). Although previous studies have shown that PBR could be the target of several anti or pro-apoptotic drugs (rasagiline, platelet-activating factor, porphyrins), indicating that the role of PBR as a modulator of mitochondrial apoptosis (143-145). PBR could regulate apoptosis through indirect effect on mitochondrial metabolism. PBR also interacts with several mitochondrial protein, VDAC and ANT, members of PTPC (146). Thus, by this direct interaction or by indirect interaction with other members, it could modify the molecular dynamics of intra-PTPC protein interactions (inhibiting for example the interaction of pro-apoptotic proteins as cyclophilinD/ANT or Bax/VADC/ANT).

4. Perspectives and open questions

In apoptotic cascades, mitochondria has been described as a check point for relaying and amplifying many of the cell death signals (147). Although some of the mechanistic details remain unclear, the finding that a pathogenic virus targets mitochondrial proteins to inhibit or induce apoptosis, as reviewed before, provides valuable tools for a better understanding of the participation of mitochondria in the regulation of cellular death.

Furthermore, increase of knowledge of physiopathological mechanism of viral infection and apoptosis modulation will constitute a promising objective to design and develop new therapies for human disease. Viral mitochondrial proteins have been shown to either protect (vMIA) from or induce apoptosis (Vpr, Hbx, M11L). Indeed, interaction of M11L and PBR is required for the lethal development of myxomatosis. Moreover, vpr mutations which abolish the apoptogenic Vpr/ANT interaction, can be found

among long term nonprogressors, correlating with decrease in apoptosis induction by HIV-1 (148, 149). For instance, drugs or peptides inhibiting the interaction of M11L, HBx, or Vpr with PTPC members could be used to neutralize their effect apoptosis and limiting viral infection. CMV is a herpes virus that can be the cause of mortality in immunodeficient patients, as individuals with AIDS or organs transplant recipients (for review (116)). As described previously in this review, vMIA can target mitochondria where it has been associated with ANT and possess an anti-apoptotic function. Moreover, infection of cells with vMIA-deficient CMV is not productive and induces apoptosis in these cells. Taken together these observations establish the possibility that drugs inhibiting ANT/vMIA interaction could restore apoptosis killing the infected cells and affecting the infection.

Legende-figure

Figure 1. Experimental methods to identify physical and functional interactions of proteins and agents with the PTPC members. ANT, purified from rat heart mitochondria or PTPC, purified from rat heart mitochondria, are reconstituted into phosphatidyl/cardiolipin liposomes where 4-methylumbelliferyl phosphate (MUP) was encapsulated. The conversion into a non-specific pore was measured by the release of MUP which reacts with alkaline phosphatase and to generate a fluorescent signal. Moreover, ANT-dependent translocase activity can be measured using ATP-preloaded ANT-proteoliposomes. These proteoliposomes are incubated with ADP and the ATP released was quantitatively measured by a luciferase chemoluminescence assay. ANT or PTPC were incorporated in planar lipid bilayers to measure their electrophysiological properties such as single channel activity or macroscopic conductance, opening frequency and ionic specificity.

Figure 2. Scheme of the putative PTPC members.

Figure 3. Putative mechanisms of Bax to induce outer mitochondrial rupture.

A. During apoptosis, cytosolic Bax conformationally changes and under Bid effect can translocate and multimerize in the outer membrane where it forms large channels which can lead the release of cytochrome *c*. B. Bax and VDAC together form a novel channel which permits the cytochrome *c* release. C. Bax may regulate opening of PTPC via an interaction with ANT.

Figure 4. Time course effect of etoposide on HeLa cells. HeLa cells were treated with etoposide 100 μ M and stained with Hoechst and JC-1. The loss of mitochondrial membrane potential is indicated by the progressive loss of the red-J-aggregate and cytoplasmic diffusion of green monomer fluorescence following exposure to etoposide. 12h after treatment, etoposide induces apoptosis in HeLa cells as shown by depolarization of mitochondrial membrane, nuclear condensation (after 20h of treatment) and apoptotic body formation (after 36h of treatment).

Figure 5. Possible mechanisms of MMP induced by viral proteins targeting the PTPC compounds VDAC, ANT and PBR. HBx translocates to mitochondria interacting with VDAC3. Vpr interacts with ANT and induces MMP. vMIA interacts with ANT (but not with VDAC) and prevents MMP. M11L is physically associated with the mitochondrial PBR component of the PTPC.

References

1. Costantini, P., Jacotot, E., Decaudin, D. & Kroemer, G. (2000) *J. Natl. Cancer Inst.* **92**, 1042-1053.
2. Kroemer, G. & Reed, J. (2000) *Nat Med* **6**, 513-9.
3. Marzo, I., Brenner, C., Zamzami, N., Jurgensmeier, J. M., Susin, S. A., Vieira, H. L. A., Prevost, M. C., Xie, Z., Matsuyama, S., Reed, J. C. & Kroemer, G. (1998b) *Science* **281**, 2027-2031.
4. Brenner, C., Cadiou, H., Vieira, H. L., Zamzami, N., Marzo, I., Xie, Z., Leber, B., Andrews, D., Duclohier, H., Reed, J. C. & Kroemer, G. (2000) *Oncogene* **19**, 329-36.
5. Costantini, P., Belzacq, A. S., Vieira, H. L., Larochette, N., de Pablo, M. A., Zamzami, N., Susin, S. A., Brenner, C. & Kroemer, G. (2000) *Oncogene* **19**, 307-14.
6. Vieira, H. L., Belzacq, A. S., Haouzi, D., Bernassola, F., Cohen, I., Jacotot, E., Ferri, K. F., El Hamel, C., Bartle, L. M., Melino, G., Brenner, C., Goldmacher, V. & Kroemer, G. (2001) *Oncogene* **20**, 4305-16.
7. Jacotot, E., Ravagnan, L., Loeffler, M., Ferri, K. F., Vieira, H. L., Zamzami, N., Costantini, P., Druillennec, S., Hoebeke, J., Briand, J. P., Irinopoulou, T., Daugas, E., Susin, S. A., Cointe, D., Xie, Z. H., Reed, J. C., Roques, B. P. & Kroemer, G. (2000) *J Exp Med* **191**, 33-46.
8. Jacotot, E., Ferri, K. F., El Hamel, C., Brenner, C., Druillennec, S., Hoebeke, J., Rustin, P., Metivier, D., Lenoir, C., Geuskens, M., Vieira, H. L., Loeffler, M., Belzacq, A. S., Briand, J. P., Zamzami, N., Edelman, L., Xie, Z. H., Reed, J. C., Roques, B. P. & Kroemer, G. (2001) *J Exp Med* **193**, 509-520.
9. Goldmacher, V. S., Bartle, L. M., Skaletskaya, A., Dionne, C. A., Kedersha, N. L., Vater, C. A., Han, J., Lutz, R. J., Watanabe, S., McFarland, E. D., Kieff, E. D., Mocarski, E. S. & Chittenden, T. (1999) *Proc Natl Acad Sci U S A* **96**, 12536-41.
10. Jan, G., Belzacq, A. S., Haouzi, D., Rouault, A., Metivier, D., Kroemer, G. & Brenner, C. (2002) *Cell Death Differ* **9**, 179-88.
11. Belzacq, A. S., Jacotot, E., Vieira, H. L., Mistro, D., Granville, D. J., Xie, Z., Reed, J. C., Kroemer, G. & Brenner, C. (2001) *Cancer Res* **61**, 1260-4.
12. Belzacq, A. S., El Hamel, C., Vieira, H. L., Cohen, I., Haouzi, D., Metivier, D., Marchetti, P., Brenner, C. & Kroemer, G. (2001) *Oncogene* **20**, 7579-87.
13. Belzacq, A. S., Vieira, H. L., Verrier, F., Vandecasteele, G., Cohen, I., Prevost, M. C., Larquet, E., Pariselli, F., Petit, P. X., Kahn, A., Rizzuto, R., Brenner, C. & Kroemer, G. (2003) *Cancer Res* **63**, 541-6.

14. Verrier, F., Deniaud, A., Rincheval, V., Mignotte, B., Jan, G. & Brenner, C. (2003) *submitted*.
15. Beutner, G., Rück, A., Riede, B., Welte, W. & Brdiczka, D. (1996) *FEBS Lett.* **396**, 189-195.
16. Marzo, I., Brenner, C., Zamzami, N., Susin, S. A., Beutner, G., Brdiczka, D., Remy, R., Xie, Z. H., Reed, J. C. & Kroemer, G. (1998a) *J Exp Med* **187**, 1261-71.
17. Ruck, A., Dolder, M., Wallimann, T. & Brdiczka, D. (1998) *FEBS Letters* **426**, 97-101.
18. Marzo, I., Brenner, C., Zamzami, N., Jurgensmeier, J. M., Susin, S. A., Vieira, H. L., Prevost, M. C., Xie, Z., Matsuyama, S., Reed, J. C. & Kroemer, G. (1998b) *Science* **281**, 2027-31.
19. Brullemans, M., Helluin, O., Dugast, J. Y., Molle, G. & Duclohier, H. (1994) *Eur Biophys J* **23**, 39-49.
20. Marzo, I., Brenner, C., Zamzami, N., Susin, S. A., Beutner, G., Brdiczka, D., Remy, R., Xie, Z. H., Reed, J. C. & Kroemer, G. (1998a) *Journal of Experimental Medicine* **187**, 1261-1271.
21. Belzacq, A. S., Vieira, H. L., Kroemer, G. & Brenner, C. (2002) *Biochimie* **84**, 167-76.
22. Zoratti, M. & Szabo, I. (1995) *Biochim Biophys Acta* **1241**, 139-76.
23. Shimizu, S., Narita, M. & Tsujimoto, Y. (1999) *Nature* **399**, 483-487.
24. Crompton, M. (1999) *Biochem J* **341**, 233-249.
25. Woodfield, K., Ruck, A., Brdiczka, D. & Halestrap, A. P. (1998) *Biochem J* **336**, 287-90.
26. Bernardi, P., Broekemeier, K. M. & Pfeiffer, D. R. (1994) *J Bioenerg Biomembr* **26**, 509-17.
27. Ichas, F., Jouaville, L. & Mazat, J. (1997) *Cell* **89**, 1145-53.
28. Bernardi, P., Colonna, R., Costantini, P., Eriksson, O., Fontaine, E., Ichas, F., Massari, S., Nicolli, A., Petronilli, V. & Scorrano, L. (1998) *Biofactors* **8**, 273-81.
29. Szabo, I., Bernardi, P. & Zoratti, M. (1992) *J Biol Chem* **267**, 2940-6.
30. Zamzami, N., Marchetti, P., Castedo, M., Zanin, C., Vayssière, J.-L., Petit, P. X. & Kroemer, G. (1995) *J. Exp. Med.* **181**, 1661-1672.
31. De Giorgi, F., Lartigue, L., Bauer, M. K., Schubert, A., Grimm, S., Hanson, G. T., Remington, S. J., Youle, R. J. & Ichas, F. (2002) *Faseb J* **16**, 607-9.
32. Lemasters, J., Nieminen, A., Qian, T., Trost, L. & Herman, B. (1997) *Mol Cell Biochem* **174**, 159-65.
33. Dorner, A., Schulze K, M., Rauch, U. & Schultheiss, H. P. (1997) *Mol Cell Biochem* **174**, 261-269.
34. Friberg, H. & Wieloch, T. (2002) *Biochimie* **84**, 241-50.
35. Bribes, E., Galiegue, S., Bourrie, B. & Casellas, P. (2003) *Immunol Lett* **85**, 13-8.

36. McEnery, M., Snowman, A., Trifiletti, R. & SH., S. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3170-74.
37. Papadopoulos, V., Dharmarajan, A. M., Li, H., Culty, M., Lemay, M. & Sridaran, R. (1999) *Biochem Pharmacol* **58**, 1389-93.
38. Crompton, M., Barksby, E., Johnson, N. & Capano, M. (2002) *Biochimie* **84**, 143-52.
39. Halestrap, A. P., McStay, G. P. & Clarke, S. J. (2002) *Biochimie* **84**, 153-66.
40. Vieira, H. L., Haouzi, D., Hamel, C. E., Jacotot, E., Belzacq, A. S., Brenner, C. & Kroemer, G. (2000) *Cell Death Differ* **7**, 1146-1154.
41. O'Gorman, E., Beutner, G., Dolder, M., Koretsky, A. P., Brdiczka, D. & Wallimann, T. (1997) *FEBS Lett* **414**, 253-7.
42. Cao, G., Minami, M., Pei, W., Yan, C., Chen, D., O'Horo, C., Graham, S. H. & Chen, J. (2001) *J Cereb Blood Flow Metab* **21**, 321-33.
43. Capano, M. & Crompton, M. (2002) *Biochem J* **367**, 169-78.
44. Shimizu, S., Ide, T., Yanagida, T. & Tsujimoto, Y. (2000) *J Biol Chem* **275**, 12321-5.
45. Kroemer, G., Zamzami, N. & Susin, S. A. (1997) *Immunol Today* **18**, 44-51.
46. Kroemer, G. (1997) *Nat Med* **3**, 614-20.
47. Adams, J. M. & Cory, S. (1998) *Science* **281**, 1322-6.
48. Vander Heiden, M. G. & Thompson, C. B. (1999) *Nat Cell Biol* **1**, E209-E216.
49. Antonsson, B., Conti, F., Ciavatta, A., Montessuit, S., Lewis, S., Martinou, I., Bernasconi, L., Bernard, A., Mermoud, J. J., Mazzei, G., Maundrell, K., Gambale, F., Sadoul, R. & Martinou, J. C. (1997) *Science* **277**, 370-2.
50. Schendel, S. L., Xie, Z., Montal, M. O., Matsuyama, S., Montal, M. & Reed, J. C. (1997) *Proc Natl Acad Sci U S A* **94**, 5113-8.
51. Spector, M. S., Desnoyers, S., Hoepfner, D. J. & Hengartner, M. O. (1997) *Nature* **385**, 653-6.
52. Pan, G. H., Humke, E. W. & Dixit, V. M. (1998) *FEBS Letters* **426**, 151-154.
53. Hengartner, M. (1997) *Nature* **388**, 714-5.
54. Chinnaiyan, A., Chaudhary, D., O'Rourke, K., Koonin, E. & Dixit, V. (1997) *Nature* **388**, 728-729.
55. Basanez, G., Nechushtan, A., Drozhinin, O., Chanturiya, A., Choe, E., Tutt, S., Wood, K. A., Hsu, Y., Zimmerberg, J. & Youle, R. J. (1999) *Proc Natl Acad Sci U S A* **96**, 5492-7.

56. Khaled, A. R., Kim, K., Hofmeister, R., Muegge, K. & Durum, S. K. (1999) *Proc Natl Acad Sci U S A* **96**, 14476-81.
57. Gross, A., Jockel, J., Wei, M. C. & Korsmeyer, S. J. (1998) *Embo J* **17**, 3878-85.
58. Wei, M. C., Lindsten, T., Mootha, V. K., Weiler, S., Gross, A., Ashiya, M., Thompson, C. B. & Korsmeyer, S. J. (2000) *Genes Dev* **14**, 2060-71.
59. Eskes, R., Desagher, S., Antonsson, B. & Martinou, J. C. (2000) *Mol Cell Biol* **20**, 929-35.
60. Antonsson, B., Montessuit, S., Lauper, S., Eskes, R. & Martinou, J. C. (2000) *Biochem J* **345**, 271-8.
61. Jurgensmeier, J. M., Xie, Z. H., Deveraux, Q., Ellerby, L., Bredesen, D. & Reed, J. C. (1998) *Proceedings of the National Academy of Sciences of the United States of America* **95**, 4997-5002.
62. Oltvai, Z. N., Milliman, C. L. & Korsmeyer, S. J. (1993) *Cell* **74**, 609-19.
63. Mikhailov, V., Mikhailova, M., Pulkrabek, D. J., Dong, Z., Venkatachalam, M. A. & Saikumar, P. (2001) *J Biol Chem* **201**, 20.
64. Murphy, K. M., Streips, U. N. & Lock, R. B. (2000) *J Biol Chem* **275**, 17225-8.
65. Cheng, E. H., Levine, B., Boise, L. H., Thompson, C. B. & Hardwick, J. M. (1996) *Nature* **379**, 554-6.
66. Zha, H. & Reed, J. C. (1997) *J Biol Chem* **272**, 31482-8.
67. O'Connor, L., Strasser, A., O'Reilly, L. A., Hausmann, G., Adams, J. M., Cory, S. & Huang, D. C. (1998) *Embo J* **17**, 384-95.
68. Huang, D. C. & Strasser, A. (2000) *Cell* **103**, 839-42.
69. Muchmore, S. W., Sattler, M., Liang, H., Meadows, R. P., Harlan, J. E., Yoon, H. S., Nettesheim, D., Chang, B. S., Thompson, C. B., Wong, S.-L., Ng, S.-H. & Fesik, S. W. (1996) *Nature* **381**, 335-341.
70. Chou, J. J., Li, H., Salvesen, G. S., Yuan, J. & Wagner, G. (1999) *Cell* **96**, 615-24.
71. McDonnell, J. M., Fushman, D., Milliman, C. L., Korsmeyer, S. J. & Cowburn, D. (1999) *Cell* **96**, 625-634.
72. Schendel, S. L., Montal, M. & Reed, J. C. (1998) *Cell Death and Differentiation* **5**, 372-380.
73. Korsmeyer, S. J., Wei, M. C., Saito, M., Weiler, S., Oh, K. J. & Schlesinger, P. H. (2000) *Cell Death Differ* **7**, 1166-73.
74. Saito, M., Korsmeyer, S. J. & Schlesinger, P. H. (2000) *Nat Cell Biol* **2**, 553-555.
75. Antonsson, B., Montessuit, S., Sanchez, B. & Martinou, J. C. (2001) *J Biol Chem* **276**, 11615-23.

76. Nechushtan, A., Smith, C. L., Lamensdorf, I., Yoon, S. H. & Youle, R. J. (2001) *J Cell Biol* **153**, 1265-76.
77. Martinou, I., Desagher, S., Eskes, R., Antonsson, B., Andre, E., Fakan, S. & Martinou, J. C. (1999) *Journal of Cell Biology* **144**, 883-889.
78. Finucane, D. M., Waterhouse, N. J., Amarante-Mendes, G. P., Cotter, T. G. & Green, D. R. (1999) *Exp Cell Res* **251**, 166-174.
79. von Ahsen, O., Renken, C., Perkins, G., Kluck, R. M., Bossy-Wetzel, E. & Newmeyer, D. D. (2000) *J Cell Biol* **150**, 1027-36.
80. Kuwana, T., Mackey, M. R., Perkins, G., Ellisman, M. H., Latterich, M., Schneiter, R., Green, D. R. & Newmeyer, D. D. (2002) *Cell* **111**, 331-42.
81. Mignotte, B. & Vayssiere, J. L. (1998) *Eur J Biochem* **252**, 1-15.
82. Fleury, C., Mignotte, B. & Vayssiere, J. L. (2002) *Biochimie* **84**, 131-41.
83. Gottlieb, E., Vander Heiden, M. G. & Thompson, C. B. (2000) *Mol Cell Biol* **20**, 5680-5689.
84. Kane, D. J., Sarafian, T. A., Anton, R., Hahn, H., Gralla, E. B., Valentine, J. S., Ord, T. & Bredesen, D. E. (1993) *Science* **262**, 1274-7.
85. Hockenbery, D. M., Oltvai, Z. N., Yin, X. M., Milliman, C. L. & Korsmeyer, S. J. (1993) *Cell* **75**, 241-51.
86. Tyurina, Y. Y., Tyurin, V. A., Carta, G., Quinn, P. J., Schor, N. F. & Kagan, V. E. (1997) *Arch Biochem Biophys* **344**, 413-23.
87. Satoh, T., Enokido, Y., Aoshima, H., Uchiyama, Y. & Hatanaka, H. (1997) *J Neurosci Res* **50**, 413-20.
88. Shimizu, S., Matsuoka, Y., Shinohara, Y., Yoneda, Y. & Tsujimoto, Y. (2001) *J Cell Biol* **152**, 237-50.
89. Madesh, M. & Hajnoczky, G. (2001) *J Cell Biol* **155**, 1003-15.
90. Sugiyama, T., Shimizu, S., Matsuoka, Y., Yoneda, Y. & Tsujimoto, Y. (2002) *Oncogene* **21**, 4944-56.
91. Brustovetsky, N. & Klingenberg, M. (1994) *J Biol Chem* **269**, 27329-36.
92. Teodoro, J. G. & Branton, P. E. (1997) *J Virol* **71**, 1739-46.
93. Afonso, C. L., Neilan, J. G., Kutish, G. F. & Rock, D. L. (1996) *J Virol* **70**, 4858-63.
94. Brun, A., Rivas, C., Esteban, M., Escibano, J. M. & Alonso, C. (1996) *Virology* **225**, 227-30.
95. Wang, G. H., Garvey, T. L. & Cohen, J. I. (1999) *J Gen Virol* **80**, 2737-40.

96. Bellows, D. S., Chau, B. N., Lee, P., Lazebnik, Y., Burns, W. H. & Hardwick, J. M. (2000) *J Virol* **74**, 5024-31.
97. Marshall, W. L., Yim, C., Gustafson, E., Graf, T., Sage, D. R., Hanify, K., Williams, L., Fingerroth, J. & Finberg, R. W. (1999) *J Virol* **73**, 5181-5.
98. Meseda, C. A., Arrand, J. R. & Mackett, M. (2000) *J Gen Virol* **81**, 1801-5.
99. Cheng, E. H., Nicholas, J., Bellows, D. S., Hayward, G. S., Guo, H. G., Reitz, M. S. & Hardwick, J. M. (1997) *Proc Natl Acad Sci U S A* **94**, 690-4.
100. Henderson, S., Huen, D., Rowe, M., Dawson, C., Johnson, G. & Rickinson, A. (1993) *Proc Natl Acad Sci U S A* **90**, 8479-83.
101. Hickish, T., Robertson, D., Clarke, P., Hill, M., di Stefano, F., Clarke, C. & Cunningham, D. (1994) *Cancer Res* **54**, 2808-11.
102. Dumont, A., Hehner, S. P., Hofmann, T. G., Ueffing, M., Droge, W. & Schmitz, M. L. (1999) *Oncogene* **18**, 747-57.
103. Han, J., Modha, D. & White, E. (1998) *Oncogene* **17**, 2993-3005.
104. Sundararajan, R. & White, E. (2001) *J Virol* **75**, 7506-16.
105. Nava, V. E., Cheng, E. H., Veluona, M., Zou, S., Clem, R. J., Mayer, M. L. & Hardwick, J. M. (1997) *J Virol* **71**, 4118-22.
106. Derfuss, T., Fickenscher, H., Kraft, M. S., Henning, G., Lengenfelder, D., Fleckenstein, B. & Meinl, E. (1998) *J Virol* **72**, 5897-904.
107. Ciminale, V., Zotti, L., D'Agostino, D. M., Ferro, T., Casareto, L., Franchini, G., Bernardi, P. & Chieco-Bianchi, L. (1999) *Oncogene* **18**, 4505-14.
108. D'Agostino, D. M., Ranzato, L., Arrigoni, G., Cavallari, I., Belleudi, F., Torrisi, M. R., Silic-Benussi, M., Ferro, T., Petronilli, V., Marin, O., Chieco-Bianchi, L., Bernardi, P. & Ciminale, V. (2002) *J Biol Chem* **277**, 34424-33.
109. Danen-van Oorschot, A. A., van Der Eb, A. J. & Noteborn, M. H. (2000) *J Virol* **74**, 7072-8.
110. Brown, J., Higo, H., McKalip, A. & Herman, B. (1997) *J Cell Biochem* **66**, 245-55.
111. Goldberg, B. & Stricker, R. B. (1999) *Immunol Lett* **70**, 5-8.
112. Kruman, II, Nath, A. & Mattson, M. P. (1998) *Exp Neurol* **154**, 276-88.

113. Schuler, W., Wecker, K., de Rocquigny, H., Baudat, Y., Sire, J. & Roques, B. P. (1999) *J Mol Biol* **285**, 2105-17.
114. Jacotot, E., Ravagnan, L., Loeffler, M., Ferri, K. F., Vieira, H. L., Zamzami, N., Costantini, P., Druillenec, S., Hoebeke, J., Briand, J. P., Irinopoulou, T., Daugas, E., Susin, S. A., Cointe, D., Xie, Z. H., Reed, J. C., Roques, B. P. & Kroemer, G. (2000) *J Exp Med* **191**, 33-46.
115. Goldmacher, V. S., Bartle, L. M., Skaletskaya, A., Dionne, C. A., Kedersha, N. L., Vater, C. A., Han, J. W., Lutz, R. J., Watanabe, S., Cahir McFarland, E. D., Kieff, E. D., Mocarski, E. S. & Chittenden, T. (1999) *Proc Natl Acad Sci U S A* **96**, 12536-41.
116. Goldmacher, V. S. (2002) *Biochimie* **84**, 177-85.
117. Benn, J. & Schneider, R. J. (1994) *Proc Natl Acad Sci U S A* **91**, 10350-4.
118. Bergametti, F., Prigent, S., Lubber, B., Benoit, A., Tiollais, P., Sarasin, A. & Transy, C. (1999) *Oncogene* **18**, 2860-71.
119. Qadri, I., Ferrari, M. E. & Siddiqui, A. (1996) *J Biol Chem* **271**, 15443-50.
120. Qadri, I., Conaway, J. W., Conaway, R. C., Schaack, J. & Siddiqui, A. (1996) *Proc Natl Acad Sci U S A* **93**, 10578-83.
121. Roulston, A., Marcellus, R. C. & Branton, P. E. (1999) *Annu Rev Microbiol* **53**, 577-628.
122. Terradillos, O., de La Coste, A., Pollicino, T., Neuveut, C., Sitterlin, D., Lecoeur, H., Gougeon, M. L., Kahn, A. & Buendia, M. A. (2002) *Oncogene* **21**, 377-86.
123. Schuster, R., Gerlich, W. H. & Schaefer, S. (2000) *Oncogene* **19**, 1173-80.
124. Wang, X. W., Gibson, M. K., Vermeulen, W., Yeh, H., Forrester, K., Sturzbecher, H. W., Hoeijmakers, J. H. & Harris, C. C. (1995) *Cancer Res* **55**, 6012-6.
125. Elmore, L. W., Hancock, A. R., Chang, S. F., Wang, X. W., Chang, S., Callahan, C. P., Geller, D. A., Will, H. & Harris, C. C. (1997) *Proc Natl Acad Sci U S A* **94**, 14707-12.
126. Takada, S. & Koike, K. (1990) *Jpn J Cancer Res* **81**, 1191-4.
127. Gottlob, K., Fulco, M., Levrero, M. & Graessmann, A. (1998) *J Biol Chem* **273**, 33347-53.
128. Pollicino, T., Terradillos, O., Lecoeur, H., Gougeon, M. L. & Buendia, M. A. (1998) *Biomed Pharmacother* **52**, 363-8.

129. Terradillos, O., Pollicino, T., Lecoœur, H., Tripodi, M., Gougeon, M. L., Tiollais, P. & Buendia, M. A. (1998) *Oncogene* **17**, 2115-2123.
130. Koike, K., Moriya, K., Yotsuyanagi, H., Shintani, Y., Fujie, H., Tsutsumi, T. & Kimura, S. (1998) *Cancer Lett* **134**, 181-6.
131. Su, F. & Schneider, R. J. (1997) *Proc Natl Acad Sci U S A* **94**, 8744-9.
132. Sheron, N., Lau, J., Daniels, H., Goka, J., Eddleston, A., Alexander, G. J. & Williams, R. (1991) *J Hepatol* **12**, 241-5.
133. Takada, S., Shirakata, Y., Kaneniwa, N. & Koike, K. (1999) *Oncogene* **18**, 6965-73.
134. Rahmani, Z., Huh, K. W., Lasher, R. & Siddiqui, A. (2000) *J Virol* **74**, 2840-6.
135. Waris, G., Huh, K. W. & Siddiqui, A. (2001) *Mol Cell Biol* **21**, 7721-30.
136. Lau, J. Y., Xie, X., Lai, M. M. & Wu, P. C. (1998) *Semin Liver Dis* **18**, 169-76.
137. Terradillos, O., Billet, O., Renard, C. A., Levy, R., Molina, T., Briand, P. & Buendia, M. A. (1997) *Oncogene* **14**, 395-404.
138. Everett, H., Barry, M., Lee, S. F., Sun, X., Graham, K., Stone, J., Bleackley, R. C. & McFadden, G. (2000) *J Exp Med* **191**, 1487-98.
139. Macen, J. L., Graham, K. A., Lee, S. F., Schreiber, M., Boshkov, L. K. & McFadden, G. (1996) *Virology* **218**, 232-7.
140. Everett, H., Barry, M., Sun, X., Lee, S. F., Frantz, C., Berthiaume, L. G., McFadden, G. & Bleackley, R. C. (2002) *J Exp Med* **196**, 1127-39.
141. Castedo, M., Perfettini, J. L. & Kroemer, G. (2002) *J Exp Med* **196**, 1121-5.
142. Casellas, P., Galiege, S. & Basile, A. S. (2002) *Neurochem Int* **40**, 475-86.
143. Youdim, M. B. & Weinstock, M. (2001) *Cell Mol Neurobiol* **21**, 555-73.
144. Verma, A., Facchina, S. L., Hirsch, D. J., Song, S. Y., Dillahey, L. F., Williams, J. R. & Snyder, S. H. (1998) *Molecular Medicine* **4**, 40-45.
145. Parker, M. A., Bazan, H. E., Marcheselli, V., Rodriguez de Turco, E. B. & Bazan, N. G. (2002) *J Neurosci Res* **69**, 39-50.
146. McEnery, M., Snowman, A., Trifiletti, R. & Snyder, S. (1992) *Proc Natl Acad Sci U S A* **89**, 3170-4.
147. Brenner, C. & Kroemer, G. (2000) *Science* **289**, 1150-1.

148. Somasundaran, M., Sharkey, M., Brichacek, B., Luzuriaga, K., Emerman, M., Sullivan, J. L. & Stevenson, M. (2002) *Proc Natl Acad Sci U S A* **99**, 9503-8.
149. Zhang, L., Huang, Y., Yuan, H., Tuttleton, S. & Ho, D. D. (1997) *Virology* **228**, 340-9.

Protein	Virus	Mecanism/action	Ref
Mitochondrial modulators			
Apoptin	Chiken anemia virus	Pro-apoptotic	109
E6	Human papilloma virus	Cell death sensitizer	110
Tat	Human immunodeficiency virus	Pro-apoptotic	111,112
p13II	HTLV-1	Pro-apoptotic	107-109
Bcl-2 homologue			
BHRF1	Epstein Barr virus	Anti-apoptotic Bcl-2 homologue	100-102
E1B19K	Adenovirus	Anti-apoptotic Bcl-2 homologue	102,103
KVS-Bcl-2	Herpes virus saimiri	Anti-apoptotic Bcl-2 homologue	105,106
Binding to PTPC members			
vMIA	Cytomegalo virus	Anti-apoptotic	115
M11L	Myxoma virus	Anti-apoptotic	138,139
HBx	Hepatitis B virus	Pro-apoptotic	133,134
Vpr	Human immunodeficiency virus	Pro-apoptotic	7,8

Table1. Viral proteins implicated in mitochondrial apoptosis

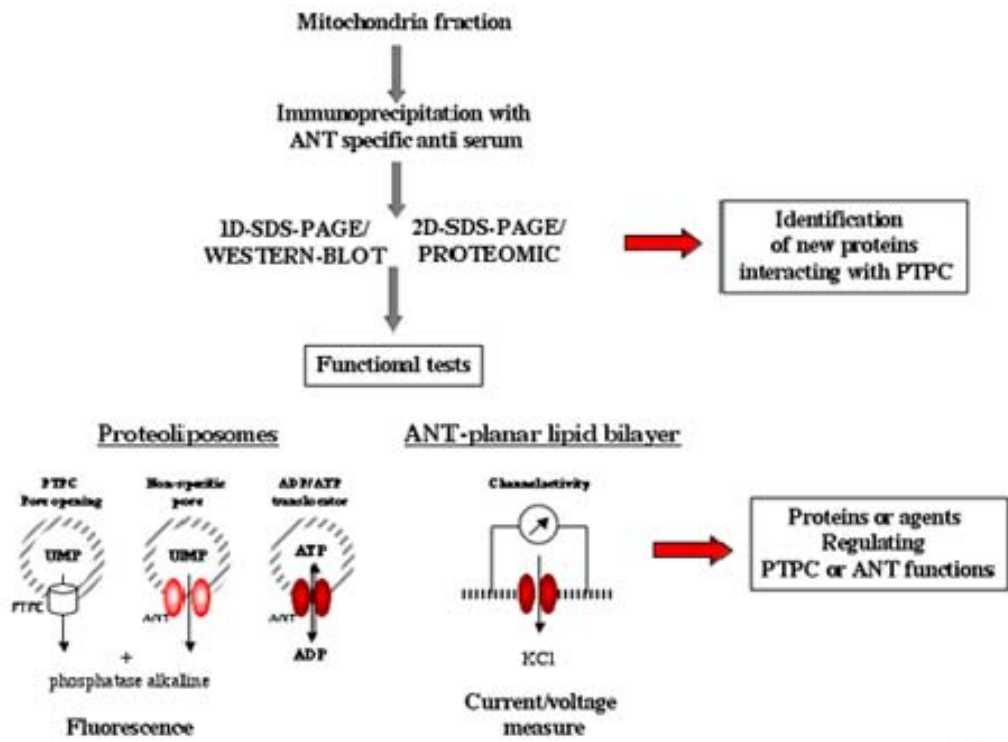


Figure 1

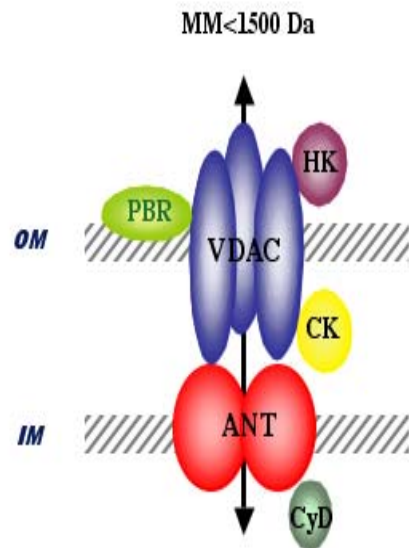


Figure 2

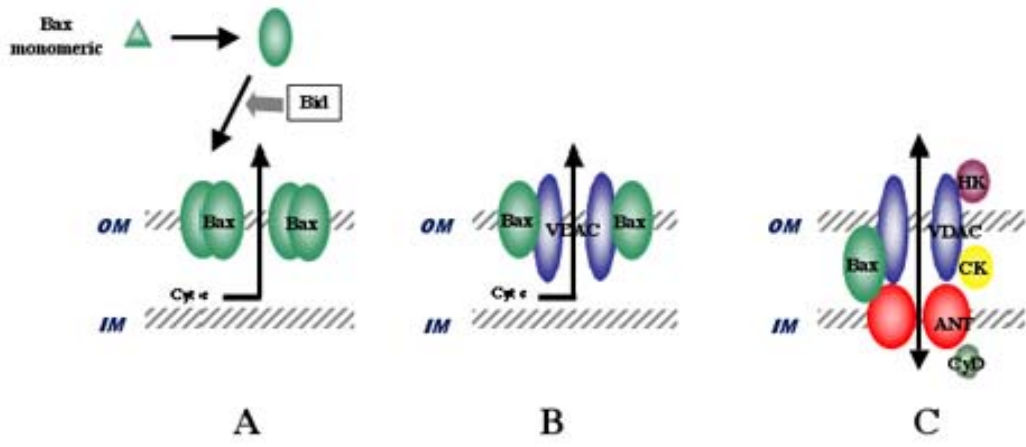


Figure 3

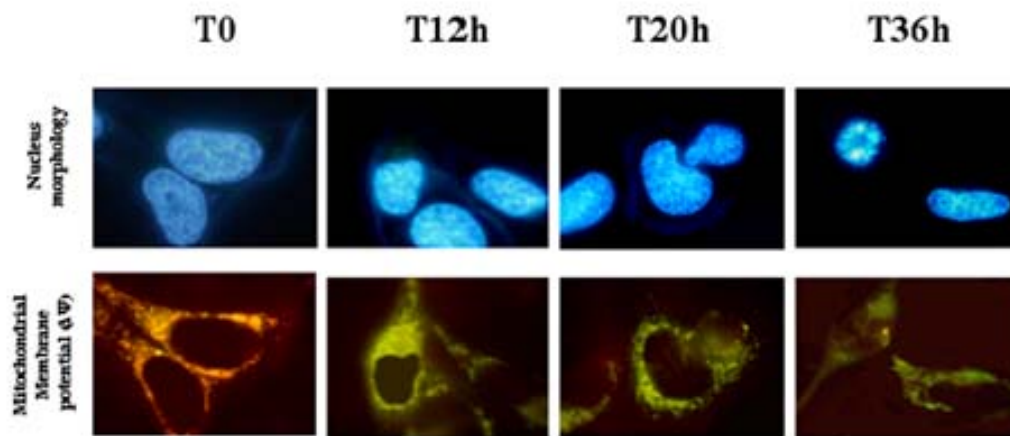


Figure 4

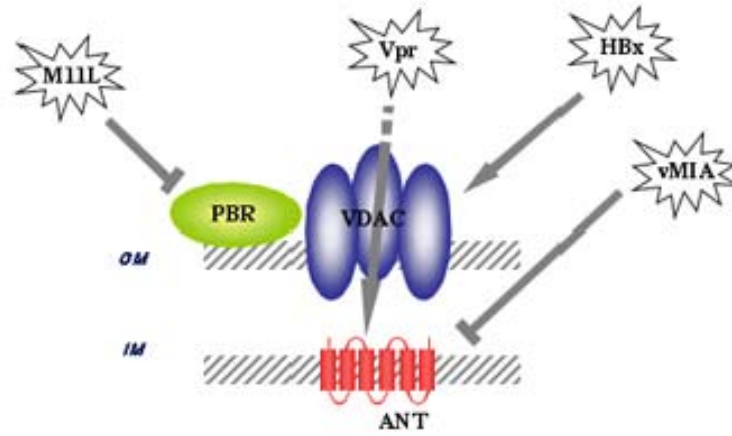


Figure 5