

On-line review: Life, death, and mitochondria

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The mitochondrial permeability transition

In recent years, it has become clear that mitochondria have a significant role to play not only in routine energy metabolism, but also in cell death (for reviews, see [1], [2], [3], [Cai, 1998 #632]. In fact, Loeffler and Kroemer [4] have suggested that apoptosis be seen as occurring in three phases: premitochondrial, mitochondrial, and postmitochondrial. During apoptosis (programmed cell death) mitochondria show a reduction in inner transmembrane potential [5], increased permeability of the outer membrane [6], and an increase in production of superoxide anions [7]. Even with pathways that may avoid direct mitochondrial initiation (i.e., the Fas pathway), mitochondria may participate in and amplify the death signal.

This role in apoptosis involves a phenomenon called the mitochondrial permeability transition, an increase in permeability that seems to be linked to the opening of a channel termed the PTP, or permeability transition pore. During the MPT, mitochondria become permeable to anything smaller than 1500 kDa, and the opening of even a single pore may be sufficient to produce the loss of membrane potential that accompanies MPT [8].

The PTP is voltage dependent, remaining closed at high membrane potentials and opening with depolarization [9]. There is, however, evidence for both a high and low-conductance state for the channel, with the pH-dependent low conductance state allowing diffusion of small ions such as Ca^{2+} [10]. One of the primary factors responsible for opening of the PTP appears to be calcium; other inducers and inhibitors may produce their effects indirectly through alteration of calcium levels [11]. Oxidation of either an "S site" by oxidized glutathione or a "P site" by oxidized pyridine nucleotides can also increase likelihood of channel opening [12], with other chemicals can affecting opening and closing of the channel either through direct effects on oxidation state of these sites, or indirectly, by altering availability of oxidized glutathione or pyridine nucleotides. Given

this, it is not surprising that conditions of oxidative stress tend to promote the MPT [8]. A third redox-sensitive site may also exist [13].

In terms of inhibition, PTP opening is inhibited at low mitochondrial matrix pH (protonation of histidyl residues keeps the channel closed) [14]. Adenine nucleotides are also strong inhibitors of PTP opening, with ADP producing a stronger effect than ATP or AMP [8]. This probably involves the adenine nucleotide translocator (ANT), and the direction it faces. MPT inhibitors such as ADP or bongkrekic acid may lock the adenine nucleotide translocator into an unfavorable conformation facing the matrix, while MPT inducers such as carboxyatractylate may lock it into a favorable, cytosol-facing conformation [15]. Cross-linking of the ANT by copper and ortho-phenanthroline has also been shown to induce the MPT [16]

There are a number of known other known inhibitors and inducers of the MPT. Cyclosporin A, for example, inhibits opening of the PTP, possibly by blocking the effects of a MPT inducer, cyclophilin D [17]. Tamoxifen has also been reported to block the MPT [18], as have a variety of quinones [19]. These compounds also inhibit the loss of membrane potential that accompanies the transition. A number of factors are also known to induce the mitochondrial permeability transition, including inorganic phosphate, retinoic acid [20], betulinic acid [21], paclitaxel (an antimicrotubule agent) [Andre, 2000 #681] and protoporphyrin IX. Pore opening is also promoted by increased electron flux through complex I [19] [19].

The permeability transition pore itself is probably created from multiprotein ensembles at the inner/outer membrane contact sites of mitochondria. Molecules involved may include the peripheral benzodiazepine receptor (from the outer membrane), a voltage-dependent anion channel (outer membrane), hexokinase and creatine kinase (intermembrane proteins), the adenine nucleotide translocator (inner membrane), porin, and cyclophilin D (matrix) [8] [22]. The participation of complex I has also been suggested [11]. The pore may span the membrane at Hackenbrock's contact sites [23].

Bcl-2 (ced-9), bcl-x_L, and bax

The bcl-2 family of proteins have been shown to play a significant role in numerous cellular processes (for review, see [24] or [25]).

The *bcl-2* protein itself (corresponding to the *ced-9* protein in *C. elegans*) is located on membranes, including the outer mitochondrial membrane, the endoplasmic reticulum, and the nuclear membrane where the C terminus is incorporated into the membrane and the N terminus faces the cytosol [26]. In terms of structure, it bears a strong similarity to the pore-forming domains of diphtheria toxin [27]. In fact, evidence indicates that Bcl-2 in the endoplasmic reticulum decreases calcium concentrations within the organelle, perhaps through increasing calcium permeability of the membrane [28]. Activation of the *bcl-2* gene, leading to expression of the *bcl-2* protein has been shown to be important in blocking apoptosis, although neither apoptosis triggered by chain blockers [29] nor by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [30] can be blocked by this protein. Expression has also been shown to block necrosis [31]. Bcl-2 has also been shown to block generation and effects of oxygen radicals [32]. The mechanism of these inhibitions may lie in the ability of *bcl-2* to block release of cytochrome c from mitochondria [6].

Bcl-2 has been shown to prolong cell cycle, increasing doubling time in transfected breast cancer cells, potentially explaining the association of its expression with favorable prognosis [33]. Bcl-2 is cleaved by caspase-3/ CPP32, and the fragment increases the sensitivity to cell death-inducing drugs [34].

Cells that overexpress *bcl-2* seem to derive most of their ATP by the process of glycolysis [35], although this metabolic effect is reversible. In addition, although withdrawal of IL-3 normally leads to metabolic arrest, it does not seem to do so in these cells. Cells that overexpress *bcl-2* will depolarize following treatment with uncouplers, and even show some cytochrome c release [36]. These cells, however, do not undergo apoptosis. Depletion of glutathione, however, not only removed the resistance to apoptosis, but could independently trigger apoptosis, indicating that redox state may play a role in sensitivity to apoptosis.

Within the Bcl-2 family, *bcl-xL* also represses apoptosis. Bcl-xL has been shown to block cell death initiated by cytochrome c addition, perhaps by inhibition of Apaf-1 (one of three identified apoptotic protease activation factors [37]). Bcl-xL has been shown to be overexpressed in cisplatin-resistant cancer cells [38]. Hsp70, however, is not induced in *bcl-xL* overexpressing cells [39].

Bcl-xS and bad do not seem to initiate, but seem to promote cell death induced through other pathways [40]. Although Bcl-xS can bind to the antiapoptotic members of the bcl family, this binding does not seem to be responsible for the promotion of cell death [40].

Bax, another apoptosis-inducing protein, may act through discharging inner transmembrane potential and inducing release of cytochrome c from mitochondria. These effects do not require protein synthesis, but can be blocked by bcl-xL or by cyclosporin A [41]. Redistribution of Bax from cytosol to mitochondria (which can be inhibited by Bcl-2) is necessary in order for the molecule to induce apoptosis [42]. Bax may interact with a permeability transition pore complex molecule called ANT (the inner transmembrane protein ATP/ADP carrier) to increase mitochondria permeability [43]. Higher levels of bax expression in tumor cells have been associated with an improved prognosis in patients with pancreatic cancer [44] and epithelial ovarian cancer [45].

In terms of regulatory control, overexpression of p53 may lead to down regulation of bcl-2. This expression appears to be hormone-dependent, with expression increasing at mid menstrual cycle and decreasing towards the end of the cycle in breast tissue [46]. Interestingly, bcl-2 and bcl-x levels are depressed in brains from Down's Syndrome patients, while bax levels were elevated [47].

Posttranslational modification of bcl-2 proteins through phosphorylation may also regulate their activity. Exposure to all trans retinoic acid has been shown to lead to serine phosphorylation of bcl-2, possibly affecting its ability to form homo and heterodimers [48]. Phosphorylation may also regulate activity that does not involve dimerization [49]. This correlates with observations that treatment of acute myeloblastic leukemia cells with all trans retinoic acid increases their sensitivity to cytosine arabinoside, an apoptosis-inducing agent [50].

Consequences of the MPT

The MPT leads to a number of deleterious effects for cell survival. Among these is the release of soluble factors (including cytochrome c) that may activate caspases (for review, see [51]). The release of cytochrome c, a diffusible carrier of electrons in the intermembrane space, into the cytoplasm may be necessary for the initiation of apoptosis

[6], and some think the MPT to be the rate-limiting event of apoptosis [52]. Interestingly, evidence suggests that in the presence of ATP (necessary for apoptosis) the MPT leads to apoptosis, while in the absence of ATP the MPT leads to necrosis [53] [54]. Clearly, discharge of the mitochondrial gradient also leads to ATP depletion and increased production of superoxide anions. Increase in cytosolic calcium [55] and decrease in pH [56] also seems to follow the MPT. Neither reduced membrane potential nor cytochrome c release may necessarily commit a cell to apoptosis, however [57]

Changes in the plasma membrane also follow the MPT. Phosphatidyl serine, normally found in the inner face of the membrane, becomes exposed on the outer membrane early in the apoptotic process, preceding changes in mitochondrial membrane potential [58]

Evidence exists that in isolated brain mitochondria, Ca^{2+} is capable of stimulating the release of cytochrome c through an alternative mechanism not involving the MPT [59].

The role of cytochrome c

Cytochrome c is coded for by a nuclear gene. Following its synthesis, the apo-cytochrome c molecule is transported into the mitochondria, where further modifications (including addition of a heme group) take place.

Evidence continues to mount for an important role for cytochrome c in apoptosis. For example, apoptosis has been shown to follow electroporation of cytochrome c into murine IL-3-dependent cells [60]. However, in the nematode *C. elegans*, cytochrome c is not required for activation of the complex consisting of CED-9 (a protein similar to Bcl-2), CED-4 (a protein similar to Apaf-1) and CED-3 (a caspase) [61]. Evidence does, however, exist that the tumor suppressor protein p53 induces apoptosis through inducing release of cytochrome c [62]. Some studies have pointed to a two-stage release of cytochrome c, with an early caspase-independent release followed by a later, larger caspase-dependent release [63]. Evidence also indicates that aspirin and other non-steroidal anti-inflammatory drugs induce apoptosis through induction of cytochrome c release [64]. Glutathione depletion, also, can initiate cytochrome c release [65].

Interestingly, the heme group seems to be required for cytochrome c activation of caspases, as apo-cytochrome c cannot carry out this function.[6].

Interestingly, in apoptotic cells, some studies have suggested that not all mitochondria seem to undergo cytochrome c release. Instead, a subset retains cytochrome c, and might be expected to provide the energy required for completion of the apoptotic program [66]. Other studies have indicated that the release of cytochrome c, once initiated, continues in a steady fashion until all cytochrome c has been released from all mitochondria within the cell [67]. Studies have also shown that embryos lacking cytochrome c are resistant to apoptosis [68]

Also, the importance of cytochrome c release to the completion of the apoptotic program has been questioned. Some researchers hypothesize that the “death commitment” is actually downstream of cytochrome c release [69].

Stimulation of CD95 (APO-1/Fas; a member of the TNF receptor subfamily) results either in the formation of a death-inducing signaling complex (DISC) which also involves MORT-1 and caspase-8, or in the activation of a ceramide-mediated apoptotic pathway [70, 71] [72]. Raisova, et.al. have shown that resistance of melanoma cells to CD95-induced cell death is likely to involve failure to release cytochrome c [73].

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