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Mini-Review

Pathophysiology of mitochondrial cell death control

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Abstract. Mitochondria have been recently recognized to play a major role in the control of apoptosis or programmed cell death. Permeabilization of mitochondrial membranes, a decisive feature of early cell death, is regulated by members of the Bcl-2 family which interact with the permeability transition pore complex (PTPC). Thus, the cytoprotective oncoprotein Bcl-2 stabilizes the mitochondrial membrane barrier function, whereas the tumor suppressor protein Bax permeabilizes mitochondrial membranes. The regulation of membrane permeabilization is intertwined with that of the bioenergetic and redox functions of mitochondria. The implications of alterations in the composition of the PTPC and in mitochondrial function for the pathophysiology of cancer (reduced apoptosis) and neurodegeneration (enhanced apoptosis) are discussed.

Key words. apoptosis; necrosis; permeability transition.

Normal control of apoptosis: the permeability transition pore complex and soluble mitochondrial intermembrane proteins

The hypothesis that mitochondria control cell death has passed through successive phases of neglect, disdain, suspicion, and (partial) acceptance [1]. Although the details are still controversial, it is agreed that in response to most proapoptotic signal transduction pathways or lethal damage pathways, mitochondrial membrane permeability is compromised, leading to the disruption of essential mitochondrial functions and to the release of soluble mitochondrial intermembrane proteins (SIMPs). Among these SIMPs, several have potential apoptogenic properties: apoptosis-inducing factor (AIF), because it can translocate to the nucleus where it causes chromatin condensation and large-scale (50 kbp) DNA fragmentation; cytochrome *c* because it interacts with Apaf-1 to activate caspase-9; heat shock protein (hsp) 10 and 60 because they facilitate the activation of caspase-3 and -9; diverse procaspases (2, 3 and 9) because they participate in proteolytic destruction; and a novel mitochondrial protease because it converts xanthine dehydrogenase into the reactive oxygen species (ROS) generator xanthine oxidase [2–10]. If these proteins, in particular caspases, become activated, they give rise to typical apoptotic cell death with oligonucleosomal DNA fragmentation, cellular shrinkage, and the formation of apoptotic bodies. In contrast, when caspases are inhibited (or when their activation is prevented due to depletion of the Apaf-1 cofactor ATP), cells can die in response to proapoptotic signals without acquiring the full apoptotic morphology [11–13]. In such a case, cells succumb to a bioenergetic catastrophe resulting from mitochondrial dysfunction and/or to caspase-independent pathways, including AIF-mediated large-scale DNA fragmentation [7].

What determines cell death is thus not only the action of SIMPs but also the underlying cause of SIMP release, mitochondrial membrane permeabilization (and, by extension, the premitochondrial triggers of this per-

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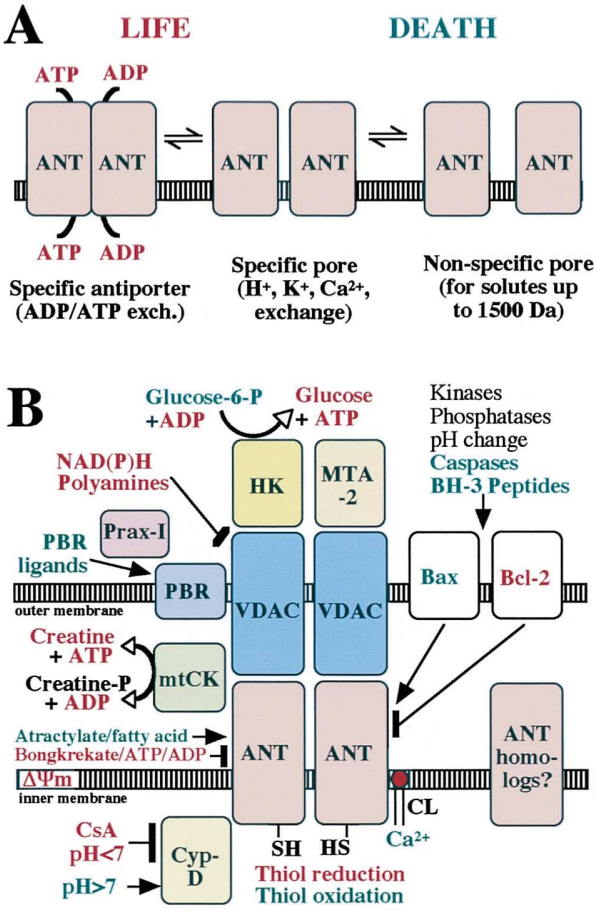

Figure 1. The permeability transition pore complex (PTPC) and its regulation. (*A*) Hypothetical dual function of the adenine nucleotide translocator (ANT). The ANT has the vital role of exchanging ADP and ATP on the inner mitochondrial membrane, functioning as a highly specific antiporter. However, ANT can also become a pore acting at several levels of conductance, specificity, and reversibility. (*B*) The PTPC in the contact site between the inner and outer mitochondrial membrane. The complex involves several transmembrane proteins [ANT, voltage-dependent anion channel (VDAC), peripheral benzodiazepine receptor (PBR), members of the Bax/Bcl-2 family], as well as associated proteins from different compartments [Prax-I, hexokinase (HK), microtubule-associated protein-2 (MTA-2), mitochondrial creatine kinase (mtCK), and cyclophilin D (Cyp-D)]. Essential reactions catalyzed by enzymes are shown. Agents or metabolites labeled in green favor PT pore opening; agents in red inhibit pore opening. Ca^{2+} may act on ANT-associated cardiolipin (CL) molecules. It is possible that, in addition to ANT, other proteins from the mitochondria carrier family participate in the PTPC. One interesting property of the PTPC is that, once activated, inner and/or outer membrane permeabilization will compromise the bioenergetic state of the cell [e.g., by causing NAD(P)H and glutathione oxidation, ATP depletion, $\Delta\Psi_m$ decrease], affect intracellular ion homeostasis (e.g., by Ca^{2+} release from the matrix), and initiate catabolic processes (e.g., via caspase activation and AIF release), which can feed back on the PTPC. This has two important implications. First, PTPC opening engages in a catastrophic self-amplification process. Second, PTPC triggers characteristic biochemical changes which do not depend on the pore-triggering stimulus. Note that the exact stoichiometry and composition of the PTPC is elusive.

meabilization, not discussed here). During apoptosis, the outer mitochondrial membrane becomes permeable to proteins, perhaps due to local physical disruption [14] (which can be transient), although alternative hypotheses suggesting the formation of large pores have been advanced [15, 16]. In contrast, the inner membrane only becomes permeable to molecules up to 1500 Da, which explains why matrix proteins are retained by the inner mitochondrial membrane, while SIMPs are released through the outer membrane. These apoptotic alterations may be attributed, at least in part, to opening of the permeability transition pore complex (PTPC), a two-membrane-spanning polyprotein complex formed at the internal/external membrane contact site. One of the core proteins of the PTPC is the adenine nucleotide translocator (ANT), the most abundant protein of the inner membrane, that normally acts as a specific ATP/ADP antiporter, yet can become a non-specific pore. Thus, ANT represents a bifunctional protein which is either a vital antiporter or a lethal pore (fig. 1A). ANT can bind to the equally abundant outer membrane protein voltage-dependent anion channel (VDAC/porin) and to the matrix protein cyclophilin D [17, 18]. ANT and/or VDAC interact with proteins involved in the regulation of energy metabolism (hexokinase II, mitochondrial creatine kinase) and the peripheral benzodiazepine receptor (PBR) (fig. 1B), as well as with the proapoptotic protein Bax and the antiapoptotic protein Bcl-2 [16, 19–21]. This appears to be functionally important because the Bax-mediated release of SIMPs is inhibited by the ANT ligand bongkrekate and by cyclophilin D ligands including cyclosporin A [20–22], at least in some experimental settings. However, it is difficult to reconcile these data with the suggestion that oligomerization of Bax alone would build up channels in the outer membrane sufficiently large to release SIMPs [15]. Whether it is the outer or the inner membrane that is permeabilized first during the apoptotic process is a matter of intense, hitherto inconclusive debate [1, 23, 24], and may well depend on the cell type and the apoptosis inducer.

Most of the available data are compatible with a model according to which the PTPC simultaneously controls the permeability of the outer membrane (pore-forming protein: VDAC, perhaps in collaboration with Bax) and that of the inner membrane (pore-forming protein: ANT and/or Bax), and participates in energy metabolism (via kinases and ANT). The PTPC would constitute a regulatory crossroad in which local gradients of ATP/ADP, redox couples (e.g., reduced vs oxidized glutathione; NAD^+ vs NADH), other metabolites (e.g., glucose, creatine), ions (Ca^{2+}, Mg^{2+}), pH, and the inner transmembrane potential ($\Delta\Psi_m$) determine the probability of membrane permeabilization (fig. 1B). As a result, the PTPC is a candidate for the

so-called 'apostat,' the regulatory device that determines in which particular metabolic conditions cells will die or survive. This implies that mitochondria would have three closely connected vital functions: (i) energy metabolism; (ii) detoxification of ROS; and (iii) conservation of mitochondrial membrane integrity (presumably by PTPC inhibition) (fig. 2). Perturbation of each of these functions may have far-reaching consequences for cell survival in health and disease.

Mitochondrial defects causing excessive apoptosis: neurodegeneration

Mutations in the mitochondrial genome cause a wide array of clinically heterogeneous syndromes among which encephalopathies with apoptotic cell loss in basal ganglia are frequent [25]. Primary neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD), are often accompanied by mutations in the mitochondrial genome. In a fraction of PD patients, complex I of the respiratory chain is deficient. Similarly, a fraction of patients with familial AD bears a deficient complex IV. Transfer of such mutant mitochondrial (mt)DNA from the patients' platelets to cells lacking mtDNA yields cytoplasmic hybrids (cybrids) which are more susceptible than control cybrids to the dissipation of $\Delta\Psi_m$ by inhibitors of the respiratory chain or to elevations of cytosolic Ca^{2+}, a classical inducer of PTPC opening. Of note, the difference in baseline $\Delta\Psi_m$ between PD and control cybrids disappears when cells are treated with the PTPC inhibitor cyclosporin A, suggesting that a constitutive increase in PTPC baseline activity is occurring, presumably as a result of enhanced generation of ROS by the deficient electron transport system [26, 27].

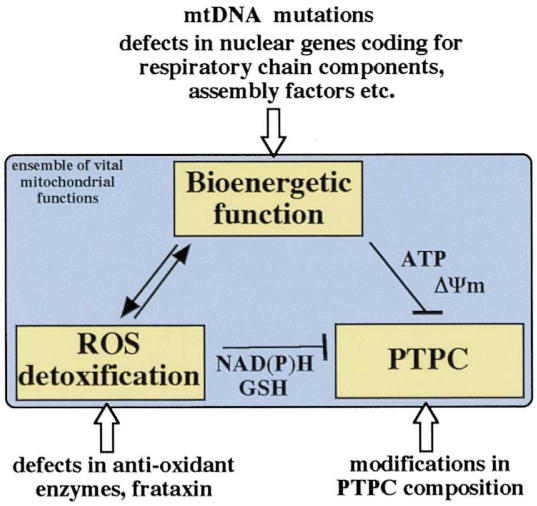

Figure 2. A triad of mitochondrial functions related to cell death control. Acquired or induced deficiencies in energy metabolism or free radical detoxification cause deinhibition of the PTPC and result in cell death. This mechanism is involved in neurodegeneration. Alterations of the composition in the PTPC (e.g., overexpression of Bcl-2-like proteins) may alter the threshold of pro-oxidant and/or distorted ADP/ATP levels that normally would cause cell death, thus facilitating the survival of cancer cells under conditions of chronic metabolic stress.

Neurodegenerative diseases are also triggered by mutations in nuclear genes encoding proteins which are imported into mitochondria. Reduced expression of frataxin, a mitochondrial protein which mediates the efflux of excessive Fe^{2+} from the matrix [28] causes Friedreich's ataxia. Knockout of mitochondrial manganese-dependent superoxide dismutase (SOD2) creates a mouse model of encephalomyopathy [25]. Mutations of the metalloprotease paraplegin, a putative cofactor of respiratory chain assembly, cause hereditary spastic paraplegia [29]. Thus, enhanced ROS generation (due to increased matrix Fe^{2+} or to a respiratory deficiency) or decreased ROS detoxification can entail neurodegeneration (fig. 2). Knockout of presenilin-1, a protein localized in the endoplasmic reticulum that is mutated in some cases of familial Alzheimer's disease, reduces the threshold of β-amyloid or Ca^{2+} required to induce a collapse of the $\Delta\Psi_m$ mediated by the PTPC [30]. Altogether, these findings indicate that perturbations of mitochondrial function, either due to mutations of mtDNA or to nuclear mutations, can play a major role in the pathogenesis of neurodegeneration.

Why do these changes, which should affect all cell types, preferentially cause apoptosis in precise areas of the brain? There are several non-exclusive answers to this question. First, neuronal cells may be particularly sensitive to perturbations of bioenergetic function, with a comparatively high threshold level below which perturbations of ATP production will cause cell death. Secondly, certain regions of the brain are characterized by increased levels of iron (substantia nigra) which may cause local oxidative stress, production of mitochondriotoxic semiquinone species due to dopamine metabolism in dopaminergic neurons, or the constitutive action of neurotransmitters that are potential excitotoxins (e.g., glutamate), also affecting mitochondrial function. Third, neuronal cells may be more susceptible than other cells to induction of PTPC opening. In vitro, neuronal mitochondria have a lower Ca^{2+}-buffering capacity than liver mitochondria [31]. Thus, general threshold effects and local imbalances may give rise to a particular regional pattern of neuronal demise accounting for the clinical specificity of each neurodegenerative disease. In accordance with this hypothesis, the Ca^{2+} sensitivity of mitochondria from three brain regions (hippocampus > cortex > cerebellum) has recently been found to correlate with the susceptibility of these regions to ischemic damage [32]. Irrespective of these

possibilities, it appears that inhibitors of the PTPC can exert neuroprotective effects, either in vitro or in vivo, as this has been shown for creatine in mice expressing mutated SOD1 [33], for overexpression of Bcl-2 in several models of acute neurodegeneration [34], and for cyclosporin A treatment in hypoglycemia [35] or traumatic brain damage [36].

Deficient apoptosis in cancer cells—a mitochondrial defect?

Cancer cells withstand an adverse microenvironment (hypoxia, acidosis, hypoglycemia, shortage of trophic supply) by virtue of a metabolic adaptation which has a profound impact on mitochondrial function. Solid tumors are characterized by a resistance to hypoxia coupled to an increased anaerobic glycolysis which is not inhibited in the presence of oxygen (Warburg effect) [37]. The hypoxia-inducible transcription factor HIF-1 and local hypoglycemia (which activates carbohydrate-responsive elements) trigger the overexpression of glucose transporters and several glycolytic enzymes. HIF-1, in conjunction with mutant p53 protein, also accounts for the overexpression of the glycolytic enzyme hexokinase II [38], which is associated with VDAC in normal brain and in a variety of tumors (but not in other normal tissues). For example, liver cell mitochondria do not bind hexokinase II, but mitochondria from hepatoma or hepatocellular carcinoma do [37], indicating that the composition of the PTPC (fig. 1B) differs between normal and malignant cells. The expression pattern of other PTPC component also changes. ANT2, which is normally repressed in quiescent cells, is expressed in dedifferentiated, proliferating tumor cells [39]. PBR, the PBR-associated protein Prax-1, and mitochondrial creatine kinase, three further putative PTPC components (fig. 1B), are also overexpressed in some tumors. Although little is known about the role of ANT isoforms or PBR in the regulation of apoptosis, it appears that mitochondrial creatine kinase can confer apoptosis resistance (and inhibition of the PTPC) in the presence of creatine [40].

Perhaps more importantly, the expression of the tumor suppressor protein Bax is frequently compromised in carcinomas, and a large portion of human tumors overexpress Bcl-2-like oncoproteins [41], emphasizing that the composition and/or control of the PTPC is drastically altered in malignancy. Bcl-2-like proteins interact with ANT, as suggested by coimmunoprecipitation and yeast two-hybrid studies [20]. Therefore, it is tempting to speculate that Bcl-2 (or other inhibitory proteins contained in the PTPC?) may act by influencing the transition of ANT from its vital (antiporter) to the lethal (pore) conformation within the PTPC (fig. 1A). Accordingly, Bcl-2 or Bcl-XL confer resistance to apoptosis induction by the ANT ligand atractyloside (which inhibits the antiporter function and favors pore formation by the ANT) [20]. Moreover, Bcl-2 and Bcl-XL protect mitochondria against membrane permeabilization due to distortion of local ATP/ADP gradients resulting from inhibition of mitochondrial F1 ATP synthases or from extramitochondrial metabolic arrest [42]. Simultaneously, Bcl-2/Bcl-XL increases the capacity of maintaining ATP/ADP exchange on the inner mitochondrial membrane in conditions of growth factor withdrawal [42]. This latter observation is in good agreement with the possibility that Bcl-2-like proteins affect the antiporter → pore transition of ANT (fig. 1A), thus underscoring the emerging connection between PTPC and cellular transformation. In contrast, the intact ATP/ADP transfer observed in Bcl-2-overexpressing cells would be incompatible with the closure of VDAC by Bcl-2 that has been postulated by Shimizu et al. [16].

Open questions and outlook

Future studies must unravel the exact composition and stoichiometry of the PTPC in normal tissues and in tumors (are their brain-specific or tumor-specific differences?), the molecular dynamics of the PTPC (what interacts with what via which domain and under which precise conditions of opening/closure?), its fine regulation by mitochondrial and extramitochondrial effectors, and its pharmacological manipulation. Which proteins within the PTPC are actually pore forming, which are mere pore regulators, and via which mechanism(s) SIMPs translocate from the intermembrane space to ectopic localizations are ongoing conundrums. If the PTPC of tumor cells is more resistant to pore opening than that of normal cells, it becomes clear why tumor cells withstand adverse metabolic conditions in which normal cells die from apoptosis. Should this speculation withstand experimental verification, then the PTPC might become a prime target for tumor-selective apoptosis induction.

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