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Review

Mitochondrial implication in apoptosis. Towards an endosymbiont hypothesis of apoptosis evolution

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Abstract

Recent evidence indicates that a profound alteration in mitochondrial function constitutes an obligatory early event of the apoptotic process. The molecular mechanism accounting for this alteration is mitochondrial permeability transition (PT). PT is both sufficient and necessary for apoptosis to occur. Experiments performed in cell-free systems of apoptosis demonstrate that mitochondria undergoing PT release protease activators that can trigger nuclear manifestations of apoptotis. Bcl-2 and its homologs are endogenous regulators of PT. It appears that some types of necrosis, those inhibited by Bcl-2, involve PT. If PT is a rate-limiting event of both apoptosis and necrosis, then downstream events including caspase activation and the bioenergetic consequences of PT must determine the choice between both modes of cell death. PT without caspase activation would cause necrosis. These findings have important implications for the comprehension of the apoptotic process, for the dichotomy between apoptosis and necrosis, and for the phylogeny of programmed cell death. Apoptosis may have evolved together with the endosymbiotic incorporation of aerobic bacteria (the precursors of mitochondria) into ancestral unicellular eukaryotes.

Keywords: mitochondrial transmembrane potential, permeability transition, programmed cell death, proteases

Abbreviations: AIF, apoptosis-inducing factor; ANT, adenine nucleotide translocator; DEX, dexamethasone; $\Delta\Psi_m$, mitochondrial inner transmembrane potential; mClCCP, carbonyl cyanide m-chlorophenlhydrazone; NGF, nerve cell growth factor; PBR, peripheral benzodiazepine receptor; PT, permeability transition; ROS, reactive oxygen species; TNF, tumor necrosis factor; VDAC, voltage-dependent anion channel

Introduction

Hundreds of different regimes induce apoptosis. In mammalian cells, such apoptotis-triggering stimuli include numerous toxins, suboptimal culture conditions, interventions on second messenger systems, and litigation of certain receptors (Fas/APO-1/CD95, TGF-R, TNF-R, etc.) or, in the case of obligate growth factor receptor, the absence of receptor occupancy (Barr and Tomei, 1994; Kroemer, 1995; Kroemer *et al*, 1995; Thompson, 1995; Wertz and Hanley, 1996). In spite of the striking heterogeneity of apoptosis induction pathways, some characteristics of the apoptotic process are near-to-constant and do not depend on the induction protocol. This applies to certain nuclear features of apoptosis such as chromatin condensation and DNA fragmentation and extends to certain plasma membrane alterations (exposure of phosphatidylserine residues on the outer leaflet), as well as cytoplasmic changes (cell shrinkage, hyperproduction of reactive oxygen species, activation of certain proteases) (Cohen, 1991; Kroemer, 1995; Kroemer *et al*, 1995; Thompson, 1995). By consequence, a common pathway of apoptosis is likely to exist. The recent discovery that programmed cell death (PCD) may be induced in anucleate cells (cytoplasts) (Jacobson *et al*, 1994; Schulze-Osthoff *et al*, 1994; Nakajima *et al*, 1995) has led to the postulation of a cytoplasmic (non-nuclear) effector or 'central executioner' that would participate in life/death decision making and would be influenced by endogenous control mechanisms (Jacobson *et al*, 1994; Oltvai and Korsmeyer, 1994; Henkart, 1995; Martin and Green, 1995). Once triggered, the central executioner would coordinate the different manifestations of the degradation phase of apoptosis, beyond the point-of-no-return of the apoptotic cascade.

The present review will summarize compelling evidence obtained by our laboratory, indicating that mitochondria play a major role in the apoptotic effector phase. In particular, I will examine the question of whether mitochondrial alterations associated with apoptotis may constitute the central executioner of cell death. Moreover, I will discuss the evolutionary implications of mitochondrial death control.

The central executioner of apoptosis: a heptalog of minimum requirements

Based on the current knowledge of apoptosis, as well as on theoretical considerations, the following seven criteria should be fulfilled by the hypothetical central executioner:

Chronological criterion

The central executioner should become activated during the effector stage of apoptosis, at the point-of-no-return, *before* the manifestations of the apoptotic degradation phase (nuclear changes, phosphatidylserine exposure, massive alterations of cellular redox potentials, advanced proteolysis of vital proteins etc.) become manifest.

Functional criterion

The central executioner should consitute an essential feature of the apoptotic process. In other terms, it should be undissociable from naturally occurring apoptosis. In addition, it should be sufficient and necessary for apoptosis to occur.

Criterion of convergence

The central executioner should be triggered by many different apoptosis induction protocols, independently from the pro-apoptotic trigger – receptor-mediated signals or damage – thus allowing for the convergence of different stimulus-dependent signal transduction pathways into one single pathway.

Criterion of coordination

The central executioner should be capable of coordinating the different manifestations of apoptosis at the levels of the nucleus, the cytoplasm, and the plasma membrane. These alterations always become apparent in a near-to-simultaneous fashion in natural apoptosis, yet can be dissociated among each other. Thus, anucleate cells manifest the cytoplasmic and plasma membrane features of apoptosis (Jacobson *et al*, 1994; Schulze-Osthoff *et al*, 1994; Nakajima *et al*, 1995), and prevention of certain cytoplasmic features (e.g. hypergeneration of reactive oxygen species) does not suppress nuclear apoptosis (Hug *et al*, 1994; Jacobson and Raff, 1995; Shimizu *et al*, 1995). The fact that certain downstream events of apoptosis can be dissociated from each other, yet occur together in most experimental setups, suggests that they are coordinated by a hierarchically superior event, namely the central executioner.

Criterion of ubiquity

All cell types can be driven to undergo apoptosis, even in the presence of protein synthesis inhibitors (Ishizaki *et al*, 1995; Weil *et al*, 1996). This indicates that mammalian cells constitutively possess all the protein and non-protein structures necessary for apoptosis, including those forming the central executioner.

Criterion of vitality

All cells, even cancer cells and transformed cell lines maintained in auxotrophic conditions, can be driven into apoptosis, provided that sufficiently drastic induction protocols are applied (Ishizaki *et al*, 1995; Weil *et al*, 1996). This implies that the apoptotic machinery including the central executioner must contain major structural elements that are essential for cell survival. (If not, cells that are completely resistant to apoptosis would be generated by mutation). The central executioner (or the compounds that compose it) must have some function(s) that is/are essential for normal cell survival.

Criterion of the switch

Cells either survive or undergo apoptosis. There is no half-way intermediate. Therefore, the central executioner should function as a switch that is either off or on. In most biological system such switches are generated by positive feedback loops (Ptashne *et al*, 1980; Scott and O'Farrel, 1986). In other words, some of the consequences that result from triggering of the executioner should themselves stimulate the executioner. Such a self-amplification pathway would constitute the simplest fashion to make the executioner react as an all-or-nothing device (Figure 1).

Mitochondrial permeability transition as a critical coordinating step of apoptosis: a few hard facts

We (Castedo *et al*, 1995; Zamzami *et al*, 1995a;b; 1996a,b; Castedo *et al*, 1996; Macho *et al*, 1996; Marchetti *et al*, 1996a,b,c; 1997; Susin *et al*, 1996a; Decaudin *et al*, 1997) and others (Vayssière *et al*, 1994; Cossarizza *et al*, 1995; Petit *et al*, 1995; Krippner *et al*, 1996; Liu *et al*, 1996; Murphy *et al*, 1996; Polla *et al*, 1996; Kluck *et al*, 1997; Yang *et al*, 1997) have recently obtained evidence indicating that mitochondrial structure and function are subject to profound

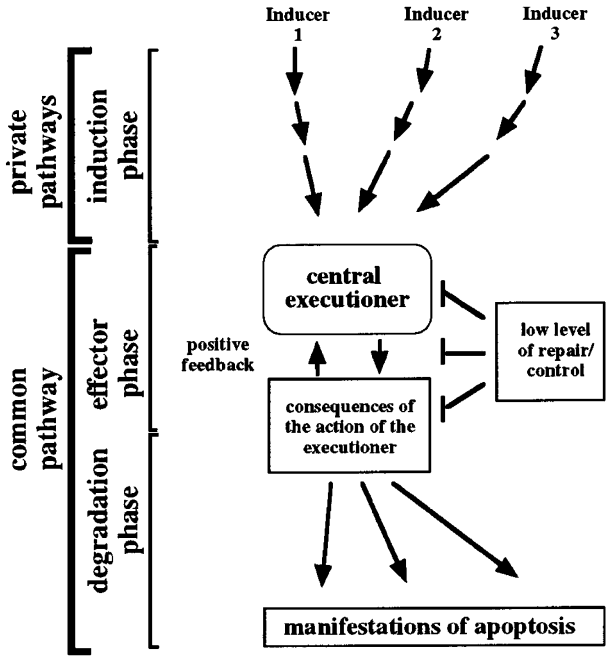

Figure 1 Schematic view of an apoptotic switch. Once induction of the central executioner is beyond the level that can be counteracted by control or repair mechanisms, the central executioner triggers one or several metabolic pathways that themselves activate the central executioner, thus engaging in a positive feedback loop. This hypothetical mechanism would explain why the central executioner would be either switched on or off instead of being activated in a gradual fashion. In addition, the diagram shows that several independent (private) pathways triggered during the apoptosis initiation phase can activate the central executioner which would constitute the bottle neck of the apoptosis process. The executioner would also be responsible for the coordination of the processes participating in the apoptotic degradation phase, beyond the point-of-no-return.

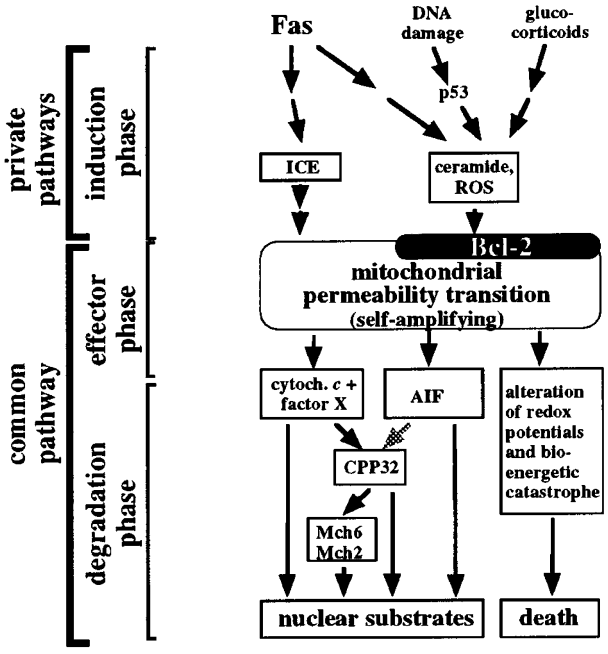

Figure 2 The apoptotic cascade. Different apoptosis-triggering pathways employ distinct signal transduction pathways that will culminate in the induction of permeability transition (PT). PT can be induced either in a Bcl-2-regulated or in a Bcl-2-independent fashion. Upon permeability transition, apoptogenic factors are released from the mitochondrial intermembrane space and leak into the cytosol. At least two such factors have been characterized: (i) cytochrome *c* (which requires unknown cytosolic factors to activate CPP32 and to induce nuclear apoptosis) and (ii) AIF which suffices to induce nuclear apoptosis *in vitro*. Different proteases from the CPP32/Ced-3 family can participate in the apoptotic degradation phase downstream of PT. In addition, PT causes major changes in cellular redox potentials (depletion of non-oxidized glutathione, hyperproduction of superoxide anion), energy metabolism (depletion of NAD(P)H_2 and ATP), and ion compartmentalization.

alterations early during the apoptotic process (reviewed by Kroemer *et al*, 1995, 1997; Marchetti *et al*, 1996d; Petit *et al*, 1996; Susin *et al*, 1996b) (Figure 2). These alterations can be attributed to mitochondrial permeability transition (PT), a well studied phenomenon that is known since the fifties (reviewed by Zoratti and Szabò, 1995; Bernardi and Petronilli, 1996). PT is a process whose physiological function has remained elusive for decades. It consists in the opening of a pore, the PT pore or mitochondrial megachannel, that can be formed by apposition of transmembrane proteins from the two mitochondrial membranes in inner/outer membrane contact sites. The exact composititon of the PT pore complex is elusive. At present, it is not known which proteins participate in its formation and which are rather involved in its regulation. One of the key elements of the PT pores is probably the adenine nucleotide translocator (ANT) (Brustovetsky and Klingenberg, 1996). The ANT has been shown to interact with outer membrane proteins including the peripheral benzodiazepin receptor (PBR, also called: endozepin receptor or receptor for the CoA-binding protein) and porin (alternative name: voltage-dependent anion channel, VDAC) (McEnergy *et al*, 1992; Kinnally *et al*, 1993). In addition, soluble proteins contained in the cytosol (hexokinase), intermembrane space (creatine kinase) and in the matrix (cyclophilin D) may participate in the formation of a dynamic multiprotein ensemble that participates in the control of the PT pore (Beutner *et al*, 1996; Nicolli *et al*, 1996) (Table 1). Some data suggest that the TIM (translocase of the inner membrane) and TOM (translocase of the outer membrane) complexes (which are involved in protein import) might also participate in the formation/regulation of the PT pore (Kinnally *et al*, 1996; Sokolove and Kinnally, 1996). It has also proposed that interactions between mitochondria and the cytoskeleton may participate in PT control (Evtodienki *et al*, 1996).

Table 1 Molecules in the PT pore complex as targets for pharmacological apoptosis modulation

Molecule (topology)	Normal function	Ligands and role in PT	Reference
Adenine nucleotide translocator (ANT) (inner membrane)	ATP/ADP antiport	Bongkrekic acid: favors m-state and inhibits PT Atractyiloside: favors c-state and induces PT	Klingenberg, 1980 Brustovetsky *et al*, 1996 Klingenberg, 1980 Brustovetsky *et al*, 1996
Peripheral benzodiazepin receptor (PBR) (outer membrane)	Receptor for endozepin (=CoA-binding protein)	Protoporphyrin IX: induces PT	Pastorino *et al*, 1994
Porin (outer membrane)	Voltage-dependent anion channel		Beutner *et al*, 1996
Cyclophilin D (matrix)	Peptidyl prolyl isomerase (chaperone function)	Cyclosporine A: inhibits interaction with inner membrane N-methyl-Val-4-cyclosporine A (non-immunosuppressive)	Nicolli *et al*, 1996
Hexokinase (cytosol)	Phosphorylates hexa-saccharides (mainly glucose) while hydrolysing ATP	Facilitates or regulates PT	Beutner *et al*, 1996
Creatine kinase (intermembrane)	Transfers phosphate from creatinephosphate to ADP or from ATP to creatine	Inhibits PT	Beutner *et al*, 1996
Matrix thiols	Thiol sensor for redox potentials	Thiol oxidation and disulfide bridge formation favor PT	Costantini *et al*, 1996
Ca^{2+} sensitive sites	Sensor for divalent cations	Calcium favors PT	Zoratti and Szabò, 1995 Bernardi and Petronilli, 1996

Numerous physiological effectors determine whether the PT pore complex adopts its open conformation. Such effectors include divalent cations (calcium, magnesium) (Szabó *et al*, 1992), protons (Bernardi *et al*, 1992), the concentration of ATP and ADP, the NAD(P)H_2/NAD(P) ratio, thiol redoxidation (Constantini *et al*, 1996), amphipathic peptides (Pfeiffer *et al*, 1995), fatty acids (Zoratti and Szabó, 1995), and perhaps calpain-like proteases (Aguilar *et al*, 1996). PT entails the free diffusion of solutes $<$1500 Da across the inner mitochondrial membrane, thus disrupting *ipso facto* the mitochondrial transmembrane potential ($\Delta\Psi_m$), which is essentially a proton gradient (Zoratti and Szabò, 1995). In addition, PT is followed by the efflux of soluble proteins from the matrix and intermembrane space (Igbavboa *et al*, 1989; Fiskum and Murphy, 1996; Susin *et al*, 1996a).

Here, I will briefly recapitulate current evidence implicating PT in apoptosis. These data have been extensively reviewed in several papers (Kroemer *et al*, 1995; 1997; Marchetti *et al*, 1996d; Petit *et al*, 1996; Susin *et al*, 1996b), which may be consulted for further details.

PT is a constant, early feature of apoptosis

In all cell types and in response to all apoptosis induction protocols tested thus far, cells manifest a collapse of the $\Delta\Psi_m$ that precedes nuclear apoptosis (Table 2). We have noted that, in contrast to one report (Yang *et al*, 1997), apoptosis induced by staurosporine, ceramide and Fas crosslinking, does involve a $\Delta\Psi_m$ disruption that precedes activation of CPP32-like caspases (Susin *et al*, 1996a and unpublished observations). PT and subsequent nuclear apoptosis are undissociable in the sense that blockade of apoptosis-inducing pathways also prevents the pre-apoptotic $\Delta\Psi_m$ disruption. We have tested specific inhibitors of PT on cells that are losing their $\Delta\Psi_m$ to show that the mechanism of the $\Delta\Psi_m$ collapse involves PT. Thus, bongkrekic acid (a ligand of the ANT), cyclosporin A (a ligand of matrix cylophilin D) and chloromethyl-X-rosamine (which acts on matrix thiols) can prevent the pre-apoptotic $\Delta\Psi_m$ loss in appropriate experimental conditions (Zamzami *et al*, 1995a; 1996a). It should be noted that cyclosporin A is only a transient ($<$1 h) inhibitor of PT (Nicolli *et al*, 1996), whereas bongkrekic acid and chloromethyl-X-rosamine are long-term PT inhibitors (Zamzami *et al*, 1995a; 1996a; Marchetti *et al* 1996a; 1997).

PT is sufficient and necessary for apoptosis

Triggering of PT with agents specifically acting on mitochondrial structures suffices to induce apoptosis (Table 2). This applies to drugs such as protoporphyrin IX (Marchetti *et al*, 1996a,b), which acts on the peripheral (mitochondrial) benzodiazepin receptor (PBR) to induce PT (Pastorino *et al*, 1994), or protonophores, which act on the inner mitochondrial membrane to disrupt the $\Delta\Psi_m$ (Zamzami *et al*, 1996b). More importantly, specific inhibitors of PT such as bongkrekic acid (Marchetti *et al*, 1996a;b; Zamzami *et al*, 1996b) and chloromethyl-X-rosamine (Marchetti *et al*, 1997) prevent PT and subsequent nuclear apoptosis in a number of different models (Table 2).

PT liberates apoptogenic factors from mitochondria

Isolated mitochondria driven to undergo PT *in vitro*, liberate at least two potentially apoptogenic factors: (i) a $\sim$50 kDa protein that we termed 'apoptosis inducing factor' (AIF) and which suffices to provoke nuclear apoptosis (chromatin condensation+oligonucleosomal DNA fragmentation) even in the absence of additional factors (Susin *et al*, 1996), and (ii) cytochrome *c* (Fiskum and Murphy, 1996), which, together with unknown cytoplasmic factors, can trigger nuclear apoptosis *in vitro* (Liu *et al*, 1996). AIF is likely to be a protease since it can be inhibited by *N*-benzyloxycarbonyl-Val-Ala-Asp.fluoromethylketone (z-VAD.fmk) (Susin *et al*, 1996a), an inhibitor of 'ICE-like' proteases which prevents apoptosis in numerous experimental models (Fearnhead *et al*, 1995; Zhu *et al*, 1995; Cain *et al*, 1996; Jacobson *et al*, 1996; Slee *et al*, 1996). Cytochrome *c* cooperates with one or several yet to be characterized cytoplasmic factor(s) to activate the protease CPP32/YAMA/Apopain (Liu *et al*, 1996), one of the signature enzymes of apoptosis (Nicholson *et al*, 1995). It remains elusive whether AIF and cytochrome *c* extrude via the PT pore or whether their liberation involves other structures in the outer mitochondrial membrane or a rupture of mitochondrial membranes.

Bcl-2 functions as an endogenous inhibitor of PT

The predominant intracellular localization of Bcl-2 and its homologs is the outer mitochondrial membrane (Krajewski *et al*, 1993; Gonzalez-Garcia *et al*, 1994). Genetic manipulations has shown that, at least in certain experimental conditions, Bcl-2 must be specifically located in mitochondrial rather than in other cellular membranes to prevent apoptosis (Tanaka *et al*, 1993; Nguyen *et al*, 1994; Zhu *et al*, 1996). In cell-free systems of apoptosis, Bcl-2 also must be present in mitochondria rather than in the nuclear envelope to inhibit apoptosis (Newmeyer *et al*, 1994; Susin *et al*, 1996a; Kluck *et al*, 1997; Yang *et al*, 1997). Bcl-2 prevents the PT-mediated $\Delta\Psi_m$ disruption in cells (Zamzami *et al*, 1995a; Castedo *et al*, 1996; Susin *et al*, 1996a; Decaudin *et al*, 1997), cytoplasts (anucleate cells) (Decaudin *et al*, 1997), and isolated mitochondria (Marchetti *et al*, 1996b; Susin *et al*, 1996; Zamzami *et al*, 1996b). Bcl-2 prevents the PT-dependent liberation of AIF from mitochondria, yet has no effect on the formation or action of AIF (Susin *et al*, 1996a). Since pharmacological inhibition of PT also prevents apoptosis (Marchetti *et al*, 1996a,b; 1997; Zamzami *et al*, 1996b), it thus appears plausible that Bcl-2 suppresses cell death via controlling PT. Recently, Bcl-2 has been shown to prevent the mitochondrial release of cytochrome *c* (Kluck *et al*, 1997; Yang *et al*, 1997). It is a matter of debate whether this is a primary effect of Bcl-2 or whether it rather results from the Bcl-2-mediated inhibition of PT. Our unpublished results, as well as those obtained by Murphy and co-workers (Fiskum and Murphy, 1996), suggest that cytochrome *c* release is a result rather than a trigger of PT. This would suggest that Bcl-2 acts at the level of PT or immediately upstream of PT. Irrespective of these details, it must be expected that

Table 2 Apoptosis-inducing regimes which induce a $\Delta\Psi_m$ disruption preceding nuclear DNA fragmentation and their inhibition

Cell type	Inducer of apoptosis	Inhibitor of apoptosis	Reference
Thymocytes	Glucocorticoids	RU38486 (receptor blockade)	Petit *et al*, 1995
			Castedo *et al*, 1995
		Actinomycin D (transcription inhibitor)	Castedo *et al*, 1996
		Cycloheximide (translocation inhibitor)	Castedo *et al*, 1996
		TLCK (trypsin inhibitor)	Marchetti *et al*, 1996a
		Z-VAD.fmk (inhibitor of proteases)	Marchetti *et al*, 1996a
		N-acetylcysteine (GSH precursor)	Zamzami *et al*, 1995b
		Catalase (H_2O_2 detoxifying enzyme)	unpub.
		Bongkrekic acid (ANT ligand)	Marchetti *et al*, 1996a
		Monochlorobiman (thiol reactive)	Marchetti *et al*, 1997
		Chloromethyl-X-rosamine (thiol reactive)	Marchetti *et al*, 1997
	Anti-Fas antibody	Ac-YVAD.cmk (ICE inhibitor)	Castedo *et al*, 1996
	DNA damage (VP16 or irrad.)	p53 null mutation	Castedo *et al*, 1996
		Actinomycin D (transcription inhibitor)	Castedo *et al*, 1996
		Cyclohexamide (translocation inhibitor)	Castedo *et al*, 1996
		TLCK (trypsin inhibitor)	Marchetti *et al*, 1996a
		Z-VAD.fmk (inhibitor of proteases)	Marchetti *et al*, 1996a
		Bongkrekic acid (ANT ligand)	Marchetti *et al*, 1996a
		Monochlorobimane (thiol reactive)	Marchetti *et al*, 1997
		Chloromethyl-X-rosamine (thiol reactive)	Marchetti *et al*, 1997
	Ceramide		unpub.
	TNFα		unpub.
	Thapsigargine		unpub.
	Ionomycine		unpub.
	Negative selection		Castedo *et al*, 1995
	Absence of positive selection		Castedo *et al*, 1995
	Menadione (ROS generator)		Castedo *et al*, 1995
	ter-butylhydroperoxide	Monochlorobiman (thiol reactive)	Marchetti *et al*, 1997
		Chloromethyl-X-rosamine (thiol reactive)	Marchetti *et al*, 1997
	H_2O_2, NO		unpub.
	Diamide	Monochlorobiman (thiol reactive)	Marchetti *et al*, 1997
		Chloromethyl-X-rosamine (thiol reactive)	Marchetti *et al*, 1997
	Protoporphyrine IX	Bongkrekic acid (ANT ligand)	Marchetti *et al*, 1996b
T cells	Glucocorticoids	Linomide (*in vivo* apoptosis inhibitor)	Zamzami *et al*, 1995b
		Bcl-2	Zamzami *et al*, 1995a
	Superantigen	Linomide (*in vivo* apoptosis inhibitor)	Zamzami *et al*, 1995b
		N-acetyl cysteine (GSH precursor)	unpub.
	Ceramide	Bcl-2 hyperexpression	Zamzami *et al*, 1995a
	ter-butylhydroperoxide	Bcl-2 hyperexpression	Susin *et al*, 1996a
	Protoporphyrine IX	Bongkrekic acid (ANT ligand)	Marchetti *et al*, 1996b
		Bcl-2 hyperexpression	Marchetti *et al*, 1996b
	mCICCP (protonophore)	Bcl-2 hyperexpression	Susin *et al*, 1996a
	Etoposide (VP16)	Bcl-2 hyperespression	Decaudin *et al*, 1997
		p53 null mutation	Castedo *et al*, 1995
	γ-irradiation	Bcl-2 hyperexpression	Decaudin *et al*, 1997
	Doxorubicin	Bcl-2 hyperexpression	Decaudin *et al*, 1997
	Cytosine arabinoside	Bcl-2 hyperexpression	Decaudin *et al*, 1997
	Staurosporine	Bcl-2 hyperexpression	unpub.
B cells	Glucocorticoids	Linomide (*in vivo* apoptosis inhibitor)	Zamzami *et al*, 1995b
	Surface IgM crosslinking	Bcl-2, Bcl-X_L hyperexpression	Decaudin *et al*, 1997
	Cyclosporin A	Bcl-2, Bcl-X_L hyperexpression	Decaudin *et al*, 1997
	Etoposide (VP16)	Bcl-2, Bcl-X_L hyperexpression	Decaudin *et al*, 1997
	γ-irradiation	Bcl-2, Bcl-X_L hyperexpression	Decaudin *et al*, 1997
	Doxorubicin	Bcl-2, Bcl-X_L hyperexpression	Decaudin *et al*, 1997
	Cytosine arabinoside	Bcl-2, Bcl-X_L hyperexpression	Decaudin *et al*, 1997
	ter-butylhydroperoxide	Bcl-2, Bcl-X_L hyperexpression	unpub.
	Ceramide		unpub.
	Serum withdrawal		unpub.
U937 cells	TNFα	N-acetylcysteine (GSH precursor)	Cossarizza *et al*, 1995
			Zamzami *et al*, 1995a
		Heat shock	Polla *et al*, 1996
	Actinomycin D	ρ^o mutation	Marchetti *et al*, 1996c
	Deoxyglucose in ρ^o cells	ρ^+ wildtype	Marchetti *et al*, 1996c
Fibroblasts	p53 release		Vayssière *et al*, 1994
Hepatocytes	TNF-α plus D-galactosamine		Zamzami *et al*, 1996b
Tumor cells	Absence of growth factors		Decaudin *et al*, 1997
	in vivo chemotherapy		Decaudin *et al*, 1997
	Staurosporine		unpub.

Table 2 *continued*.....

Table 2 *continued*

Cell type	Inducer of apoptosis	Inhibitor of apoptosis	Reference
Neurons	NGF withdrawal	NGFreaddition	Deckwerth *et al*, 1993
	Glutamate	Cyclosporin A	Ankarcrona *et al*, 1995
			Schinder *et al*, 1996
			White and Reynolds *et al*, 1996
		NMDA receptor blockade	White and Reynolds *et al*, 1996
		Absence of extracellular calcium	White and Reynolds *et al*, 1996
		Inhibitor of mit. Na^+/Ca^{2+} exchanger	White and Reynolds *et al*, 1996
	Respiratory chain inhibitors	Bcl-2 hyperexpression	Shimizu *et al*, 1996

Bax, an antagonist of Bcl-2, should induce $\Delta\Psi_m$ disruption. Indeed, transfection-enforced hyperexpression of Bax has recently been shown to provoke a collapse of the $\Delta\Psi_m$ (Xiang *et al*, 1996).

Mitochondrial permeability transition (PT): the central executioner of apoptosis?

The data discussed above indicate that mitochondria are involved in apoptosis. However, does this imply that mitochondria events control the central executioner? Here, I will examine the possibility that mitochondrial PT might constitute (or form part of) the central executioner. Does PT meet the seven minimum requirements that the central executioner should fulfil?

Chronological criterion

PT-mediated $\Delta\Psi_m$ disruption is indeed an early, pre-nuclear event of the apoptotic process (Castedo *et al*, 1995; 1996; Zamzami *et al*, 1995a,b; 1996a,b; Macho *et al*, 1996; Marchetti *et al*, 1996a,b,c; 1997; Susin *et al*, 1996a; Decaudin *et al*, 1997). Cells that have disrupted their $\Delta\Psi_m$ are irreversibly committed to undergo death, even when the apoptosis-inducing trigger is withdrawn (Zamzami *et al*, 1995a). Thus, the $\Delta\Psi_m$ collapse marks the point-of-no-return of apoptosis, yet precedes all common signs of the apoptotic degradation phase: nuclear apoptosis, PS exposure on the membrane, and activation of CPP32-related caspases.

Functional criterion

PT appears indissociable from subsequent nuclear apoptosis. It is sufficient and necessary for nuclear apoptosis to occur. This notion is based on pharmacological evidence. Induction of PT causes apoptosis, and its inhibition prevents apoptosis (Marchetti *et al*, 1996a,b; 1997b; Zamzami *et al*., 1996b). In addition, the finding that Bcl-2 and other apoptosis-inhibitory Bcl-2 homologs such as Bcl-X_L prevent PT (Castedo *et al*, 1996; Susin *et al*, 1996a; Zamzami *et al*, 1996b; Decaudin *et al*, 1997) emphasizes the functional importance of PT in life/death decision making.

Criterion of convergence

PT is triggered by numerous physiological and aphysiological effectors, both in cells and in isolated mitochondria (Table 1). Thus, it appears that numerous stress responses (increase in calcium, depletion in non-oxidized glutathione, ATP or $NAD(P)H_2$ decrease, genotoxic stress, proteases etc.) can trigger PT in cells (Table 2), rendering PT an attractive candidate for a universal stress sensor. It appears that PT is not only activated by unphysiological stress responses. Rather, it is also triggered by signal transduction pathways activated upon ligation of receptors (glucocorticoid receptor, Fas/APO-1/CD95, TNF-R etc.) as well as ceramide, a second messenger involved in several apoptotic pathways. At present, it is not known which are the final molecular effectors responsible for the induction of apoptotic PT when apoptosis is receptor-induced.

Criterion of coordination

PT entails a number of profound alterations in cell metabolism, all of which are constant features of apoptosis. In addition to provoking nuclear apoptosis (Marchetti *et al*, 1996b; Susin *et al*, 1996a; Zamzami *et al*, 1996b), PT causes an increase in superoxide generation on the uncoupled respiratory chain (Zamzami *et al*, 1995a), thereby profoundly affecting cellular redox potentials. Thus, PT entails the depletion of non-oxidized glutathione (Marchetti *et al*, 1996a; Macho *et al*, 1997) and the irreversible superoxide mediated oxidation of cardiolipins and other membrane lipids (Zamzami *et al*, 1995a,b). This observation, together with the obvious effects of PT on energy metabolism, indicates that PT triggers several pathways each of which would be lethal by itself. Pharmacological inhibition of PT prevents all manifestations of apoptosis, at the levels of the nucleus, the plasma membrane, and the cytoplasma (Marchetti *et al*, 1996a), emphasizing that PT can indeed function as a central coordinating event.

Criterion of ubiquity

Essential components of the PT such as the ANT and porin are found in all cell types. These molecules are phylogenetically old and relatively conserved. Indeed, molecules such as the ANT (which exchanges matrix ATP for cellular ADP) are essential for oxidative phosphorylation and thus must be expressed in all living cells (Klingenberg, 1980). For obvious reasons, the apoptogenic factor cytochrome *c*, which participates in the respiratory chain, also must be present in all respiring cells. AIF-like activity has also been detected in many different tissues (Susin *et al*, 1996a), suggesting that it may be ubiquitous.

Criterion of vitality

Although some if not all PT pore components are essential for cellular metabolisms and although PT is phylogenetically old – it has been described in yeast mitochondria (Szabo *et al*, 1995) – it is not clear whether PT might have any physiological function not related to apoptosis. Thus, this issue has to await future clarification. According to Brdiczka and coworkers, the PT pore might be important for the handling of ATP and metabolic control (Beutner *et al*, 1996). This idea is based on the observation that many of the proteins involved in formation/regulation of the PT pore specifically interact with ATP: porin (which regulates ATP flux through the outer membrane; Rostovtseva and Colombini, 1996), hexokinase, and obviously the ANT. Bernardi and coworkers (Bernardi and Petronilli, 1996) propose an alternative physiological role for PT. Short spikes of PT may be involved in the periodic outflow of calcium from the mitochondrial matrix and this would be necessary to avoid excessive calcium accumulation in mitochondria. Another hypothesis is brought forward by Kinnally and colleagues. These authors suggest that the PT pore might be identical with a mitochondrial multiple conductance channel modulated by peptides responsible for targeting mitochondrial precursor proteins (Kinnally *et al*, 1996; Sokolove and Kinnally, 1996). This would imply that the PT pore participates in the import of nuclear gene products into mitochondria. If one of these hypotheses was confirmed, PT would be essential for normal mitochondrial function.

Criterion of the switch

As summarized in Table 3, many of the consequences of PT themselves trigger PT. Thus, $\Delta\Psi_m$ disruption, increases in cytosolic free Ca^{2+} concentrations, oxidative changes in the redox potential, and protease activation result from PT at the time that they induce PT. This feature of self-amplification renders PT an attractive candidate to constitute the (or a) death switch.

In synthesis, it appears that PT could fulfil most if not all criteria of the central executioner. At this point, it should be noted that different proteases, mainly those from the ICE family, have also been suggested to form part of the executioner (Martin and Green, 1995; Henkart *et al*, 1996). Future investigation will have to clarify the exact relationship between PT and protease cascades, as well as their relative contribution to the central executioner of apoptosis.

The necrosis/apoptosis paradox: resolved?

Although apoptosis has been initially defined by confronting it with necrosis (Kerr *et al*, 1972), several observations question the paradigmatic opposition between these two types of cell death. As a matter of fact, several pathologies which were previously thought to involve primary necrosis, are now described to involve apoptosis: apoplexy (Martinou *et al*, 1994), myocardial infarction (Itoh *et al*, 1995), kainate-induced neural cell death (Simonian *et al*, 1996), and ischemia/reperfusion damage (Fliss and Gattinger, 1996; Sasaki *et al*, 1996). More importantly, hyperexpression of the apoptosis-inhibitory oncoprotein Bcl-2 has been shown to inhibit necrotic cell death in a number of models: kainate-induced neuronal necrosis (Kane *et al*, 1993), occlusion of the midbrain artery (Martinou *et al*, 1994), absence of oxygen (Shimizu *et al*, 1995), and chemical hypoxia (Shimizu *et al*, 1996). PC12 pheochromocytoma cells treated with rotenone or cyanide die from necrosis after having disrupted their $\Delta\Psi_m$. Transfection-enforced hyperexpression of Bcl-2 prevents both the $\Delta\Psi_m$ disruption and the subsequent necrosis (Shimizu *et al*, 1996). Pharmacological induction of PT entails hepatocyte necrosis (Pastorino *et al*, 1994; Trost and Lemasters, 1996). This suggests that, at least in some instances, PT constitutes a molecular event that is also involved in necrosis.

The above findings may be integrated into the following hypothesis (Figure 3). When PT is induced in a massive, rapid fashion heavily compromising the ATP supply, necrosis (that is primary disruption of the plasma membrane) occurs before apoptogenic proteases are activated and can act on nuclear and cytoplasmic substrates. In contrast, induction of PT in a more smooth, protracted fashion, would allow for the activation and action of specific proteases (AIF, CPP32 and other downstream proteases) before ATP depletion and other consequences of PT such as enhanced superoxide anion generation cause cell death. In other terms, the intensity of

Table 3 Self-amplifying features of mitochondrial permeability transition (PT)

Inducer of PT	Consequence of PT
$\Delta\Psi_m$ reduction facilitates PT (Bernardi, 1992)	PT causes $\Delta\Psi_m$ disruption (Zoratti and Szabò, 1995)
Calcium causes PT (Szabò *et al*, 1992; Zoratti and Szabò, 1995)	PT causes outflow of matrix calcium and ATP depletion, thereby disrupting calcium homeostaia (Zoratti and Szabò, 1995)
Thiol oxidation of matrix proteins induces PT (Costantini *et al*, 1996)	PT causes depletion of non-oxidized glutathione, thereby favoring protein thiol oxidation (Zoratti and Szabò, 1995
Oxidation of $NAD(P)H_2$ favors PT (Costantini *et al*, 1996)	PT results in $NAD(P)H_2$ oxidation (Petit *et al*, 1995)
Reactive oxygen species induce PT (Zoratti and Szabò, 1995)	PT causes hypergeneration of superoxide anion on the uncoupled respiratory chain (Vayssière *et al*, 1994; Zamzami *et al*, 1995)
Proteases can cause PT (Marchetti *et al*, 1996a)	PT results in protease activation (Lui *et al*, 1996; Susin *et al*, 1996a)

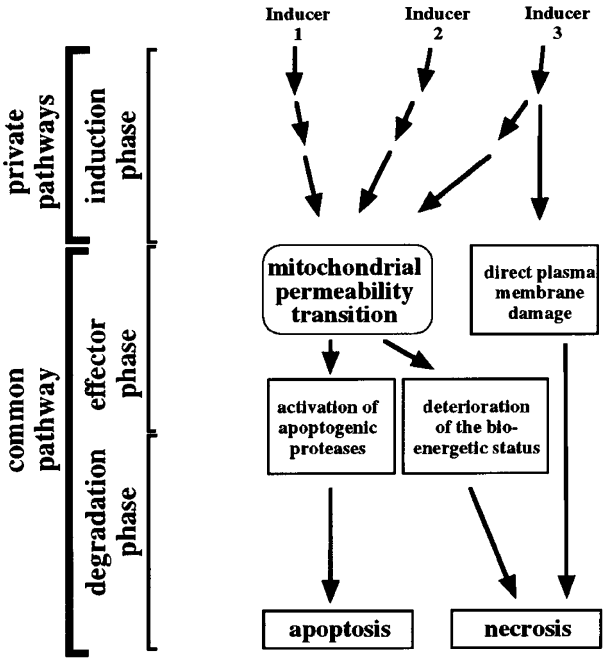

Figure 3 Hypothetical model of the dichotomy between apoptosis and necrosis. Massive induction of PT with subsequent rapid depletion of ATP causes primary necrosis, that is the disruption of plasma membrane integrity before apoptogenic proteases come into action. In contrast, a more subtle induction of PT allows for the activation of proteases culminating in manifest nuclear apoptosis. In some instances, direct plasma membrane damage provokes fulminant necrosis without that PT would be required for cell death.

the PT-inducing stimulus would determine which among two major consequences of PT wins the race: a bioenergetic and redox catastrophe culminating in necrosis or the activation/action of apoptogenic proteases. This scenario would be compatible with the finding that many drugs induce necrosis at high doses and apoptosis at lower ('subnecrotic') doses (Kroemer, 1995). It would also be compatible with the observation that modulation of ATP levels can shift the balance between apoptosis and necrosis to one or the other mode of cell death (see accompanying reviews by Nicotera and Leist, 1997; Tsujimoto, 1997). Finally, it would explain why cells in which the activation of most if not all caspases is inhibited by means of the inhibitor Z-VAD.fmk (*N*-benzyloxycarbonyl-Val-Ala-Asp-fluromethylketone) undergo non-apoptotic (necrotic?) rather than apoptotic cell death when transfection-enforced hyperexpression of the Bcl-2 antagonists Bax or Bak causes disruption of mitochondrial function (Xiang *et al*, 1996; McCarthy *et al*, 1997).

The evolutionary origin of death: premises of an endosymbiont hypothesis of apoptosis

As discussed in the foregoing sections, mitochondrial PT appears to be the decisive mechanism of apoptosis. This may have major implications for the phylogeny of apoptosis. I will first expose the premises of my hypothesis regarding the evolutionary origin of apoptosis and then formulate my speculation.

The endosymbiotic origin of mitochondria

It is widely accepted that eukaryotic cells are descendants of primitive anaerobic organisms that survived, in a world that had become rich in oxygen, by engulfing aerobic bacteria – keeping them in symbiosis for the sake of their capacity to consume atmospheric oxygen and to produce ATP. Thus mitochondria would originate from Krebs-cycle-containing eubacteria (promitochondria) invading a fermentative anaerobe (Margulis, 1975). During eukaryotic evolution, then most of the genetic information contained in the pro-mitochondrial genome has been incorporated into the nuclear genome (Gray, 1989).

Programmed cell death is phylogenetically old

Apoptosis or apoptosis-like phenomena have been described for a number of unicellular eukaryotes, including *Trypanosoma cruzi* (Ameisen *et al*, 1995), *Trypanosoma brucei rhodesiense* (Welburn *et al*, 1996), *Leishmania amazonensis* (Moreira *et al*, 1996), *Tetrahymena thermophila* (Mpoke and Wolfe, 1996) and *Euglena gracilis* (Scheuerlein *et al*, 1995). Apoptosis-like phenomena are also found in fungi (Cornillon *et al*, 1994) and plants (Mittler and Lal, 1995; Greenberg, 1996; Jones and Dangl, 1996). Thus, apoptosis as such has not evolved during the invention of multicellularity, as has been generally assumed, but rather before. Although developmentally *regulated* (*programmed*) cell death is probably only instaured in multicellular organisms (Raff, 1994), it appears that the basic mechanisms of apoptosis are already found in unicellular organisms. Thus apoptosis may have evolved at the same time as did endosymbiosis.

PT, some constituents of the PT pore and several apoptosis effector molecules are phylogenetically old

PT-like phenomena have been described in *Saccharamyces cerevisiae* (Szabo *et al*, 1995). A few molecules contained in the PT pore complex are found in both prokaryotes and eukaryotes: cyclophilins and porins (Schulz, 1996). An analog of the PBR has been described in *Rhodobacter capsulatus* (Armstrong *et al*, 1989). The ANT gene is found in the nuclear genome of unicellular eukaryotes, plants, fungi and metazoans (Kuan and Saier, 1993; Walker and Runswick, 1993), and functionally analogs may be found in *Rickettsia prowazekii* (Krause *et al*, 1985; Carmeli and Lifshitz, 1989) as well as the internal membrane of certain bacteria such as *Rhodobacter capsulatus* (Carmeli and Lifshitz, 1989). However, the mitochondrial carrier family of transport proteins, to which the eukaryotic ANT belongs, has been suggested to arise shortly *after* the formation of the endosymbiotic relationship between the progenitor of the mitochondrion and that of eukaryotic cells (Walker and Runswick, 1993). Members of the *bcl-2* gene family exhibit structural analogies with colicins (Muchmore *et al*, 1996), a family of bactericidal compounds elaborated by *Escherichia coli* (Stroud, 1995). Intriguingly, the *bcl-2* homologue of *C. elegans, ced-9*, forms part of a polycistronic locus also containing *cytochrome b540*, which belongs to respiratory chain complex II (Hengartner and

Horvitz, 1994). This may suggest that both genes have been transferred together from the pro-mitochondrial genome to the nucleus. Cytochrome *c* is already contained in aerobic bacteria, and some mitochondrial and extramitochondrial proteases may well have a bacterial origin (Rawlings and Barrett, 1993). In conclusion, it appears possible that many of the constituents of the PT pore and several apoptogenic mitochondrial proteins were already present in the aerobic bacterium from which the mitochondrion evolved.

Members of the Bcl-2 family act on an evolutionary conserved death pathway

As mentioned above, members of the Bcl-2 family of apoptosis-regulatory proteins from animals have structural equivalents in bacteria. Importantly enough, certain products of the Bcl-2 gene family are lethal across species barriers. Thus the Bcl-2 antagonist Bax (which is necessary for neuronal apoptosis to occur in mice; Knudson *et al*, 1995) kills *Saccharomyces cerevisiae* cells carrying functional mitochondria as well bacteria in which it is overexpressed (Sato *et al*, 1994; Greenhalf *et al*, 1996; Zha *et al*, 1996). To kill yeast cells, Bax must be targeted to mitochondria (Zha *et al*, 1996). Bcl-2 has also been shown to rescue mutant yeast strains with defects in superoxide dismutase from the lethal effects of growth under aerobic conditions (Kane *et al*, 1993). Moreover, it can prevent the lethal effect of Bax overexpression in yeast cells (Sato *et al*, 1994; Greenhalf *et al*, 1996; Zha *et al*, 1996). It may be important to note that yeast cells killed by Bax overexpression do not undergo oligonucleosomal DNA fragmentation nor present any signs of apoptotic morphology (chromatin condensation, membrane blebbing etc.). Thus, it appears that the Bcl-2/Bax regulation of cell death – at the level of mitochondria – is phylogenetically conserved between pluricellular animals and unicellular fungi, whereas nuclear apoptosis is not.

Addiction modules

One possibility to confer a selective replication advantage to bacterial plasmids consists in the generation of an 'addiction module' (Jensen and Gerdes, 1995; Naito *et al*, 1995; Yarmolinsky, 1995). If the plasmid encodes both a long-lived toxin and a short-lived antidote, then loss or

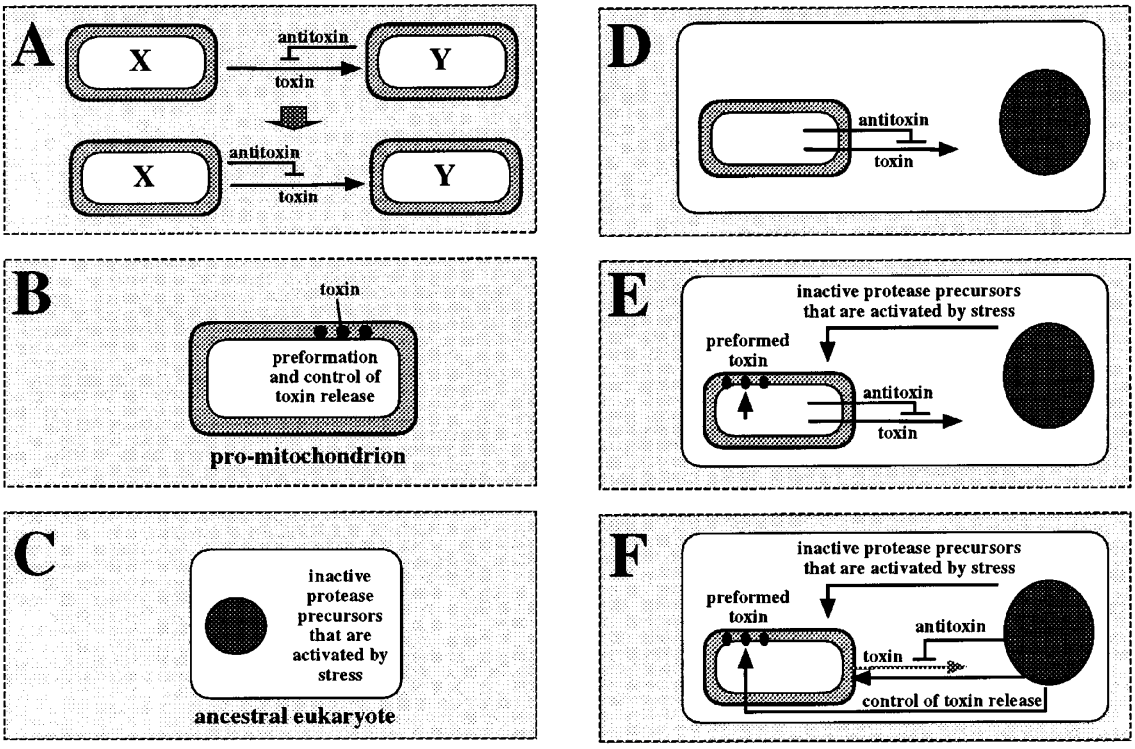

Figure 4 The endosymbiont hypothesis of apoptosis. (**A**) Generation of an addiction module in bacteria. A bacterium X generates a toxin for strain Y, which in turn produces an antitoxin. The toxin-producing strain eventually acquires the genetic information for the antitoxin. (**B**) As an alternative, a bacterium produces toxins that are pre-formed, compartmentalized and only released in particular circumstances, e.g. after aggression/lysis by other organisms. (**C**) The ancestral eukaryote still lacking mitochondria possesses proteases or other enzymes with antibiotic activity that are activated upon stress (e.g. invasion by bacteria). (**D**) Addiction in endosymbiosis. The pro-mitochondrion which incorporates into the pro-eukaryote produces both a toxin and an antitoxin. Since the latter has a shorter half life than the former, elimination of the mitochondrion would be lethal for the eukaryotic host cell. (**E**) Same as D incorporating the possibilities demonstrated in **B** and **C**. The bacterium contains pre-formed toxins that are released upon lysis of the bacterium. In addition the host produces antibiotics that are either present as inactivated precursors or a compartmentalized (e.g. in lysosomes). Any damage or stress to the host would activate or liberate these antibiotics and thus cause bacterial death which in turn would cause liberation of toxins from the bacterium. (**F**) Due to the shuffling of genes from the mitochondrial to the nuclear genome, the genetic control of most of the mechanisms described above pass to the nucleus. However, certain toxin precursors or metabolic pathways causing the production of toxins still locate to the mitochondrion.

destruction of the plasmid by a bacterium that has commenced its transcription will kill the bacterial host. Failure to transcribe the antidote-encoding gene would cause depletion of the short-lived life-saving molecule before the toxin disappears. This mechanism has been proposed to be at the origin of bacterial 'programmed cell death' (Yarmolinsky, 1995). A similar mechanism could be involved in the generation of endosymbiosis. Thus, the pro-mitochondrion invading the ancestral eukaryote might have developed one or several 'addiction modules' to stabilize the host/parasite micro-ecosystem.

The endosymbiont hypothesis of apoptosis: a speculative scenario

Based on the above premises, it is conceivable that the basic mechanism of apoptosis became fixed during evolution in the very moment in which endosymbiosis became established. Speculatively, invading aerobic bacteria may have employed suitable combinations of relatively stable toxins and labile antitoxins ('addiction modules') in order to become indispensable for the host (Figure 4A). Alternatively or in addition, such bacteria also contained pre-formed host-specific toxins so that bacterial lysis would kill the host cell (Figure 4B). This implies that bactericidal enzymes (e.g. specific proteases) produced by the host cell have to remain inactive, either by maintaining them as immature precursors or by sequestering them in subcellular compartments (e.g. lysosomes) well separated from the intruding bacterium (Figure 4C).

In this speculative scenario, a stable equilibrium based on mutual dissuasion would establish between the host and its invader. Any attempt of the pro-mitochondrion to kill the host and *vice versa* would be fatal for both partners, which thus have been trapped into symbiosis (Figure 4D, E). From this moment, the two initially independent organisms are forced to co-evolve. During this co-evolution, large parts of the bacterial genome are gradually incorporated into the nuclear genome (Figure 4F). This explains why murine cells from which the mitochondrial DNA has been depleted (and which thus possess mitochondria lacking DNA) can survive in special culture media and still can be induced to undergo apoptosis (Jacobson *et al*, 1993; Marchetti *et al*, 1996c,d). However, many of the apoptosis-controlling proteins encoded by nuclear genes continue to be imported into mitochondria (ANT, PBR, porin, cytochrome *c* etc). or to act on mitochondrial membranes (Bcl-2 and homologues).

It is posible that mitochondrial PT has evolved together with or shortly after the establishment of endosymbiosis (Figure 5). As discussed above, cyclophilin, porin/VDAC-like molecules, PBR-like molecules, and even structural Bcl-2 homologues (the colicins and diphteria toxin) are found in certain bacteria. In this context, it is intriguing that porin-like molecules may fulfil an essential role for endosymbiosis, as suggested by the fact that only porin B-expressing *Neisseria* can accommodate as intracellular pathogens in epithelial cells. Incorporation of such a *Neisseria* porin into the host phagosome membrane surrounding phagocytosed *Neisseria* is essential for intracellular parasitism, possibly due to porin's function as an ATP sensor (Rudel *et al*, 1996). It is conceivable that during the redistribution of such molecules from bacterial to host membranes, for instance during the transposition of porin from the outer bacterial membrane to host membranes, the basic PT pore became established. As an alternative, the PT pore may have arisen when the ANT evolved after the initial stage of endosymbiosis (Walker and Runswick, 1993). Intriguingly, the ANT plays a major role in the distribution of ATP between hosts and intracellular parasites in another system (*Plasmodium falciparum* in human erythrocytes) (Kanaani and Ginsburg, 1989). The essential role of the PT pore (or of its components) in the host-parastie coordination, for instance at the level of ATP metabolism or respiratory control, would then account for the fixation of PT throughout eukaryotic evolution. In other words, the interaction of a few proteins at the host/parasite interface would be neuralgic for endosymbiosis but would also lay the evolutionary grounds of apoptotic cell death. Some apoptosis effector molecules with essential metabolic functions (cytochrome *c* and perhaps proteases) were already present in the precursor of the mitochondrion. The release of these molecules would then become subordinated to PT, thereby connecting PT to the

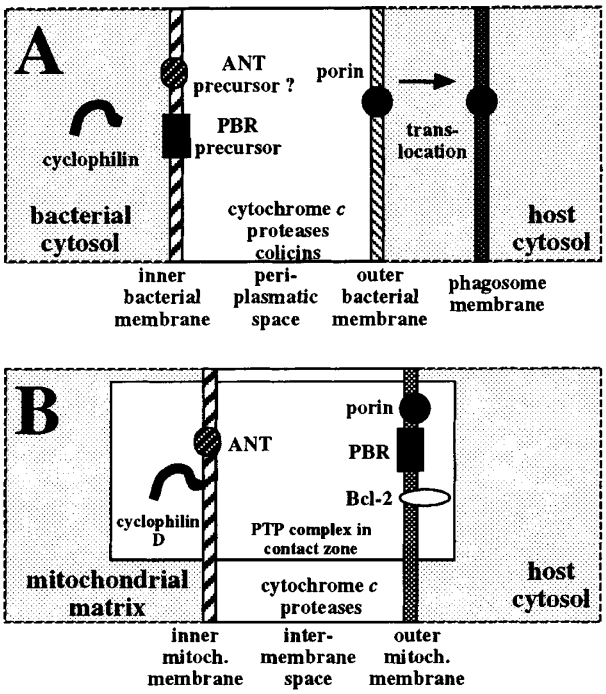

Figure 5 Highly speculative model for the molecular evolution of mitochondrial permeability transition (PT). (**A**) Hypothetical state of the PT pore precursor in the moment of bacterial pathogen accommodation. An aerobic bacterium possessing two membranes (inner + outer membrane) is surrounded by a phagosome membrane of the eukaryotic host cell. During this step, certain molecules e.g. porins translocate from bacterial to host membranes and assure the diffusion of small molecules (including ATP) at the host-parasite interface. (**B**) Later during evolution, the ANT arises in its mature form, allowing for the formation of the PT pore complex (within the white rectangle). Note that only few changes are necessary to evolve from **A** to **B**. Within the intermembrane space of bacteria and mitochondria potentially toxic molecules including cytochrome *c* and certain proteases are present. The PT-dependent release of such molecules may be ultimately responsible for apoptosis.

activation of proteases which degrade vital proteins and give rise to the apoptotic phenotype.

The endosymbiont hypothesis of apoptosis would explain why this mode of cell death already exists in unicellular eukaryotes, in which the existence of PCD obviously cannot constitute a direct advantage for Darwinian selection. In this context it should be mentioned that apoptosis induced in unicellular eukaryotes is only observed in response to non-physiological (non-receptor-mediated) triggers such as irradiation, antibiotics, complement or suboptimal culture conditions (Ameisen *et al*, 1995; Scheuerlein *et al*, 1995; Moreira *et al*, 1996; Mpoke and Wolfe, 1996; Welburn *et al*, 1996). It is plausible that *regulated* apoptosis (*programmed* cell death) has developed concomitantly with multicellularity, when PT control becomes connected with signal transduction pathways.

In addition to its heuristic value, the endosymbiont theory of apoptosis allows for the formulation of a series of testable hypotheses. Thus for instance, primitive unicellular eukaryotes still lacking mitochondria (e.g. *Giardia intestinalis*) should lack apoptosis-like phenomena. Moreover, apoptosis-like death of cells from plants and fungi should be associated with mitochondrial alterations early during the death process. Future studies will unravel these incognita.

Conclusions

Although apoptosis has been commonly described as a nuclear process, it now appears that mitochondrial permeability transition (PT) is the (or one of the) mechanisms which 'decides' between death and life. PT fulfils most if not all criteria that must be met by the central executioner of apoptosis: (i) PT is an early event of the apoptotic cascade; (ii) it is sufficient and necessary for the induction of apoptosis; (iii) it is induced by many different endogenous effectors and apoptosis induction protocols; (iv) its inhibition by specific drugs or Bcl-2 hyperexpression is sufficient to prevent all downstream manifestations of apoptosis; (v) it functions in all mammalian cell types; (vi) it may be vital for normal metabolism; and (vii) it has self-amplifying properties and thus responds in an all-or-nothing fashion.

PT has at least three lethal consequences: (i) it causes the liberation of apoptogenic proteases and protease activators from the mitochondrial intermembrane space into the cytosol; (ii) it entails the hyperproduction of superoxide anions from the uncoupled respiratory chain and thus causes a severe, irreversible derangement of the redox potential; and (iii) it provokes the depletion of energy-rich phosphates ($NAD(P)H_2$ and ATP). PT is not only essential for apoptotic cell death; its involvement in certain models of necrosis has been documented. It is possible that the pace at which PT is induced, as well as the availability of apoptogenic proteases and/or their substrates may decide the mode of cell death. The metabolic consequences of PT cause necrosis unless proteases come into action. Whenever PT is induced in a fulminant fashion and/or the energy metabolism is severely compromised, the bioenergetic and redox catastrophe following PT causes necrotic cell death before apoptosis can occur. This scenario would weaken the opposition between apoptosis and necrosis and explain how Bcl-2, which functions as an inhibitor of PT, can prevent both apoptosis and, at least in some systems, necrosis.

The mitochondrial control of apoptosis may have far-reaching implications for the evolution of cell death. It appears plausible that apoptosis arose as a by-product of endosymbiosis in unicellular eukaryotes before multicellularity developed.

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