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Mitochondria, glutamate neurotoxicity and the death cascade

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Abstract

This review focuses on two questions: the role of mitochondria in excitotoxic neuronal death and the connection of mitochondria with the apoptotic death cascade. The goal is to highlight the regulatory role of mitochondrial channels on the mitochondrial membrane potential, $\Delta\psi$, and their involvement in determining neuronal survival or death. A hypothesis is developed centered on the notion that protein–protein interactions between members of the Bcl-2 family of death suppressor and promoter proteins lead to the selective elimination of depolarizing currents that, in turn, collapse $\Delta\psi$ and set in motion the irreversible pathway of cell death. The model considers the remarkable propensity of Bcl-2 family proteins to dimerize or oligomerize and thereby restrict the localization of partner molecules to mitochondrial membrane contact sites. The fundamental principle invoked here is that through a concerted set of protein–protein interactions, information is exchanged by specific heterodimers, one of the partners acting as a toxic protein and the second as its antidote. The review concludes with the elaboration of a speculative model about cellular mechanisms for the prevention of cell destruction as triggered by extracellular signals which may be conserved in its molecular design from bacteria to eukaryotes.

Keywords: Neuronal death; Excitotoxicity; Apoptosis; Bcl-2; Ion channel; Protein–protein interaction; Signal transduction

1. Involvement of mitochondrial dysfunction in excitotoxic neuronal death

A widely held view considers excitotoxic death, the process by which prolonged activation of excitatory neurotransmitter receptors leads to neuronal death, as a pathogenic component in acute ischemic brain injury, and in the development of chronic neurodegenerative disorders which are associated with neuronal loss, such as Parkinson's disease, Huntington's disease and Alzheimer's dementia [1–5]. The underlying mechanisms for the selective vulnerability of neurons are largely unknown. Glutamate receptors of the *N*-methyl-D-aspartate (NMDA) subtype are highly permeable to Ca^{2+} and mediate its massive influx leading to an imbalance of cellular homeostasis and, in due turn, to cell death [6,7]. Collapse of the mitochondrial $\Delta\psi$ with the ensuing drop of cellular ATP, release of apoptosis inducing factors, production of reactive oxygen species and NO, and activation of numerous degradative enzymes are all events intimately involved in the derangement of cellular homeostasis triggered by unregulated glutamate [1–5]. Indeed, blockers of the NMDA receptor prevent excitotoxicity by arresting the cascade at the departure point [8].

Mitochondria are unique among cell organelles in

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their involvement on the concerted consumption of oxygen, production of oxygen radicals and mobilization of intracellular Ca^{2+} ($[Ca^{2+}]_i$) [9–15]. Given that excessive Ca^{2+} accumulation in mitochondria uncouples electron transfer from ATP synthesis and considering that impairment of energy metabolism increases generation of free radicals [14–20], mitochondria have emerged as the missing link between elevation of $[Ca^{2+}]_i$ and glutamate neurotoxicity (Fig. 1) [21].

The critical role of mitochondria in excitotoxicity has been recently documented primarily by examining the relationship between changes of $[Ca^{2+}]_i$, mitochondrial $\Delta\psi$ and neuronal cell death evoked by glutamate agonists. For the sake of brevity, this assessment is described following the pathway outlined in Fig. 1 [21]. Prolonged stimulation of NMDA receptors in cultured hippocampal [19,21], cortical [20] or cerebellar granule [15,22–26] neurons evokes a massive accumulation of Ca^{2+}, which has been estimated to reach an equivalent $[Ca^{2+}]_i$ of $\sim$20 mM [27]. Persistent elevation of $[Ca^{2+}]_i$, i.e. Ca^{2+} overload, increases with the duration of the glutamate agonist challenge. Concomitantly, the glutamate-induced Ca^{2+} overload produces neuronal death. Time-lapse fluorescence imaging of the Ca^{2+} indicator Fluo-3 AM on hippocampal neurons identified a disproportionately higher incidence of cell death than anticipated from a tight correlation with the extent of Ca^{2+} overload [21]. Such disparity suggested that additional steps intervene between elevation of $[Ca^{2+}]_i$ and cell death. Similar results were obtained by imaging cerebellar granule cells using Fura-2 AM [15,22,23] and forebrain neurons by indo-1 microfluorimetry [20].

The involvement of mitochondria was highlighted by the enhanced susceptibility to NMDA-induced Ca^{2+} overload by antimycin A and rotenone, specific inhibitors of complex III and complex I of the electron transport chain. Significantly, antimycin A [21] and cyanide, the specific inhibitor of complex IV [28], increase the susceptibility of hippocampal neurons to NMDA-induced cell death, and complex I inhibitors rotenone [29] and 1-methyl-4-phenylpyridinium [30] enhanced the vulnerability of dopaminergic neurons to NMDA-induced cell death. These results suggest a model in which mitochondrial homeostasis acts as the gatekeeper between cell survival and death (Fig. 1).

2. Excitotoxic neuronal death and the collapse of mitochondrial membrane potential

The link between NMDA receptor overstimulation, Ca^{2+} sequestration, and impairment of mitochondrial function was strengthened by measurements of mitochondrial $\Delta\psi$ during the excitotoxic challenge using fluorescent potentiometric dyes (tetramethylrhodamine ethyl ester-TMRE [21], rhodamine-123 [15,22–24], or JC-1 [20]. Glutamate agonists induce prominent mitochondrial depolarization, with a time scale coincident with the development of Ca^{2+} overload: increasingly longer NMDA stimuli induce more conspicuous depolarizations, with decreasing extents of recovery establishing a linkage between NMDA receptor overactivation and mitochondrial impairment. A tight association between the incidence of neuronal cell death and mitochondrial damage as evidenced by the extent of mitochondrial depolarization was readily established, strengthening the connection between NMDA-induced Ca^{2+} entry and collapse of $\Delta\psi$ (Fig. 1) [20,21,25,26].

It was surmised that the concurrent build-up of intramitochondrial $[Ca^{2+}]$ and attenuation of $\Delta\psi$ might induce opening of the mitochondrial inner membrane permeability transition pore (PTP), thereby allowing fluxes of ions and small molecules and collapsing $\Delta\psi$ [31–34] (Fig. 1). The PTP is the subject of other reviews in this issue. Here we focus on the specific blockade of the PTP by the immunosuppressant drug cyclosporin A (CsA) [12,33] which has provided clues to implicate the PTP in excitotoxicity [20,21,25]. If the PTP were a key entity in the cascade leading to the Ca^{2+} induced collapse of $\Delta\psi$, it would be anticipated that blocking the opening of the PTP would promote the recovery of $\Delta\psi$ and would prevent neuronal cell death. This prediction was fulfilled. Quantitative analysis of results from a large population of CsA exposed neurons showed that the recovery of the mitochondrial depolarization is practically complete, suggesting that the persistent opening of the PTP underlies the collapse of $\Delta\psi$ [21]. Remarkably, CsA protects neurons from glutamate-induced death. These results implicate the PTP in the collapse of $\Delta\psi$ elicited by prolonged activation of the NMDA receptor and establish a connection between mitochondrial dysfunction and excitotoxic neuronal death [19–26,34–36] (Fig. 1).

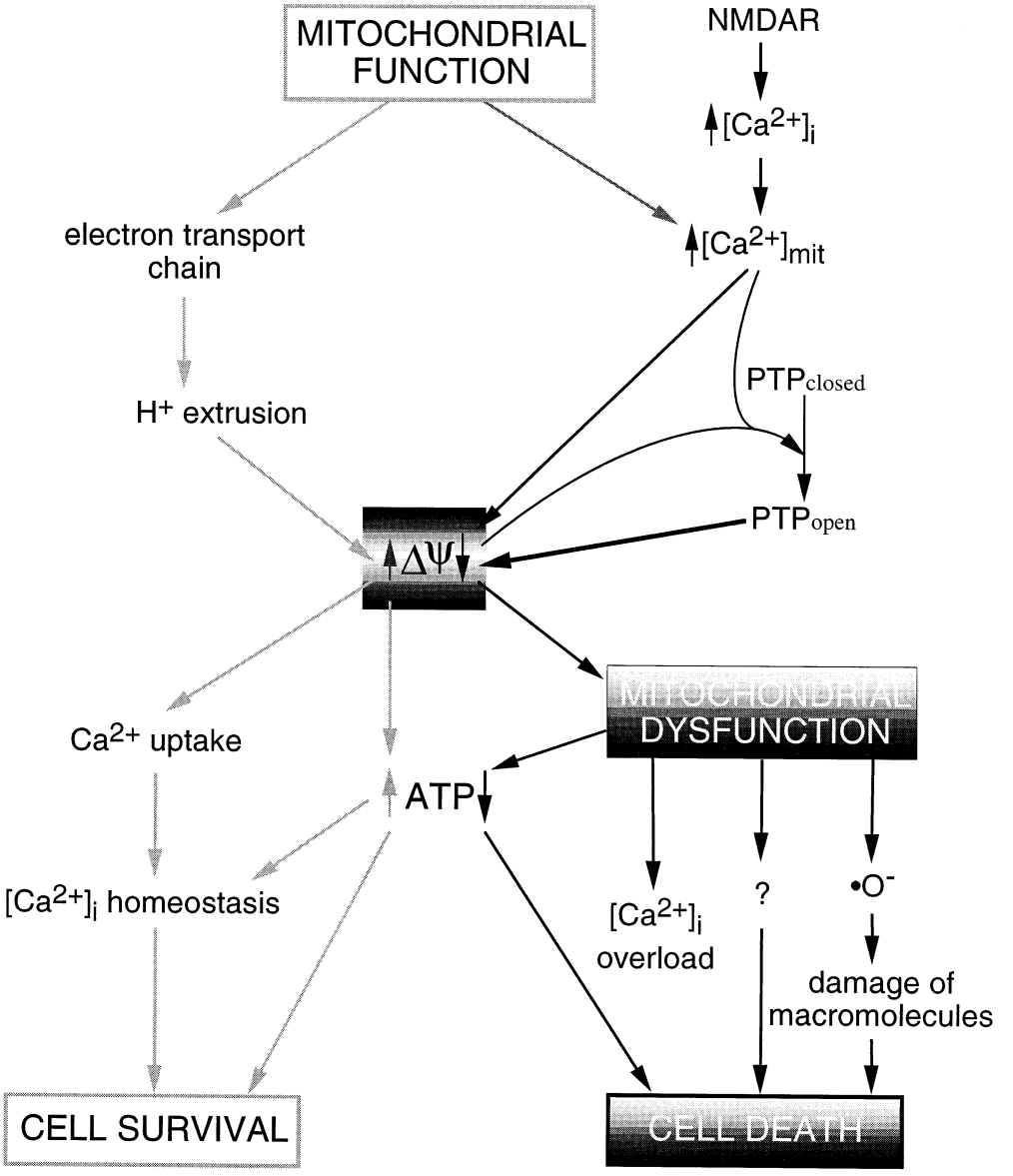

Fig. 1. Schematic representation of potential pathways by which mitochondrial dysfunction could act as an effector of excitotoxic neuronal death. Excessive activation of the NMDA receptor (NMDAR) induces massive Ca^{2+} influx and abnormal elevations of $[Ca^{2+}]_i$. Mitochondrial Ca^{2+} uptake, driven by $\Delta\psi$, attenuates $\Delta\psi$. This, in turn, causes a decrease in ATP synthesis and the opening of the PTP, which collapses $\Delta\psi$. Mitochondrial dysfunction elicits a further reduction in intracellular ATP pools, increases free radical generation, and most likely activates other processes that ultimately contribute to neuronal death (from [21]). Reproduced with permission.

It is worthy of note that mitochondrial Ca^{2+} entry is only one of the factors known to trigger the PTP [37]. Conspicuous among others are reactive oxygen species and NO [34,38,39], considered key players in the cascade of events leading to cell death. Furthermore, activation of the cytosolic phospholipase A2 (cPLA2) by Ca^{2+} overload [40] is likely to release arachidonic acid, an uncoupler of oxidative phosphorylation, with consequent mitochondrial swelling and leakage of components out from the mitochondrial matrix [34]. Significantly, mice deficient in cPLA2 exhibit reduced postischemic brain injury [41]. In concert, these factors and others, may contribute to the derangement of cellular homeostasis that ultimately leads to neuronal cell death (Fig. 1).

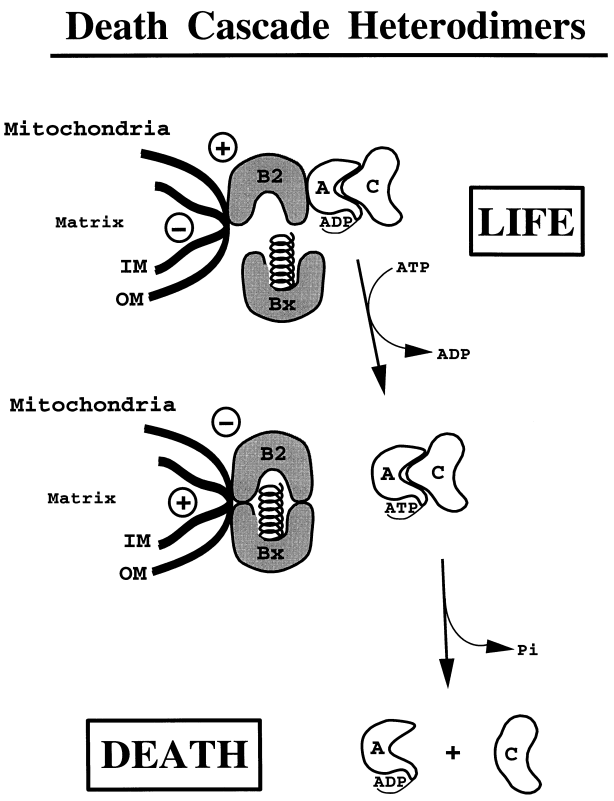

Fig. 2. Schematic representation of the information flow in a death cascade in which the signal relays are specific heterodimers, The contact sites between the inner (IM) and outer (OM) mitochondrial membranes are displayed as the site of anchoring or insertion of Bcl-2 family proteins. The + and − signs indicate the polarity of the mitochondrial membrane potential $\Delta\psi$. B2 and BX denote Bcl-2 and Bax proteins as prototypes of death suppressor and promoter members of the family. The coil protruding from the surface of BX represents the $\alpha 2$ helix that binds in a complementary cleft of Bcl-X_L. A and C represent Apaf-1 and Caspase 3, respectively (for review see [74]).

3. Connection with the apoptotic death cascade

Several pieces of information provide clues about the association of the programmed cell death pathway with excitotoxic neuronal death [1–4,26,31]. Conspicuously, the PTP-mediated collapse of $\Delta\psi$ is abrogated by the anti-apoptotic protein Bcl-2 [31,32,34,42]. This raises the possibility that Bcl-2 may be a subunit of the PTP or a modulator of the PTP. While Bcl-2 overexpression potentiates the maximum Ca^{2+} uptake in neurons and attenuates the generation of reactive oxygen species [43], Bcl-2 deficient mice show substantial degeneration of peripheral sensory and sympathetic neurons as well as motor neurons [44]. By contrast, the archetypal proapoptotic protein Bax is required for the death of sympathetic neurons and motor neurons after trophic factor deprivation [45]. Intriguingly, the Bcl-2 family proteins appear to be localized at mitochondrial membranes [46–48]. These findings, therefore, set mitochondria at the crossroads of the death cascade. What is known about the cascade and about the biological activities of these proteins?

4. Heterodimers in the death cascade

Signal transduction is the process by which an extracellular signal upon recognition by a specific surface receptor triggers a cascade of biochemical events inside the cell that lead to immediate, and long-term changes that ultimately determine the activity and fate of the cell, including, but not limited to, growth, differentiation, survival or death. These cascades involve complex networks of interacting molecules of diverse size. The majority of these molecules are proteins that act by presentation or by recognition of surface determinants which lead to specific interactions between partner molecules. Interactions then lead to the specific actions of the involved partners, frequently based on the interaction-induced conformational changes. The newly produced surfaces, in due turn, bring about new activities, for example, uncovery or hindrance of enzymatic activity, and creation or abrogation of a binding surface, or even relocation of partner molecules between intracellular compartments. Overall, these cascades exhibit the salient feature of amplification and are, therefore, involved in the regulation and control of critical cellular pathways responsible for the maintenance of cellular homeostasis. Any imbalance in these molecular circuits can induce derangements, the specificity and intensity of which may determine the ultimate fate of the cell. These cascades are robust, meaning that they display adaptation or the ability to resist perturbations.

A case in point is the process of apoptosis. The survival or death of a cell can be viewed as the expression of regulation, or dysregulation, by protein conformational dynamics. A family of proteins known as Bcl-2 proteins has been identified as key controlling elements of the apoptotic cascade [49–53].

The family includes members which act as death suppressors or anti-apopoptotic such as Bcl-2 (26 kDa) and Bcl-X_L (27 kDa), and death promoters or pro-apoptotic, such as Bax (21 kDa), Bak, Bik and Bcl-X_S. Interactions between these two classes of proteins frequently oppose their actions and their relative frequency of occurrence appears to modulate the propensity of a cell to undergo apoptotic death [49–53]. Multiple activities of these proteins have been delineated and several targets recognized. Despite the fact that the picture is still incomplete, a conspicuous characteristic of members of this family has emerged: Bcl-2 proteins exhibit a strong propensity to dimerize or oligomerize [49,50,54,55]. It is precisely this proclivity that may be at the root of their intracellular activities.

Insights into the molecular basis for the function of Bcl-2 proteins have emerged from the structure at atomic resolution of Bcl-X_L, an anti-apoptotic member of the Bcl-2 family, and its complex with a peptide derived from Bak, a proapoptotic protein. The structures were obtained by X-ray crystallography and NMR spectroscopy [56,57]. The recombinant Bcl-X_L protein used for the structural determination did not include the transmembrane C-terminal anchor. The structure shows that Bcl-X_L exhibits the globin fold: seven helices form a compact globular structure. The surface involved in dimerization has been traced to a hydrophobic groove created by four helices, namely $\alpha2$, $\alpha4$, $\alpha5$ and $\alpha7$. $\alpha2$, also known as the BH3 domain, has been recognized as a key component in all death-promoting members of the Bcl-2 family, which are also devoid of $\alpha1$, known to contain the BH4 domain [58]. Remarkably, cleavage of Bcl-2 by caspases releases the N-terminal fragment that contains the BH4 domain ($\alpha1$) and uncovers a proapoptotic activity of Bcl-2 [59]. These considerations suggest that an exposed $\alpha2$ may be required to promote death. The structure of a complex of Bcl-X_L and a 16-mer peptide patterned after the sequence of Bak $\alpha2$ was also determined. This peptide adopts an amphiphilic helical structure that binds in the cleft of Bcl-X_L through hydrophobic and electrostatic interactions. The structure of the complex indicates that the molecular basis for the dimerization involves the presentation on the surface of one partner of a complementary amphipathic helix that fits into the groove of the second partner molecule. Complemen-

Table 1
Death cascade heterodimers

Key heterodimers	Features
Bcl-2/Bax	● Mitochondrial localization ● Control of $\Delta\psi$ ● Channel formation with multiple roles: (a) release of cytochrome *c*, ATP, and other unidentified components (b) release Apaf-1 from ternary complex (c) attenuate or abolish the Bax-induced collapse of $\Delta\psi$ (d) restoration of $\Delta\psi$ removes driving force for Bax insertion into the membrane (e) exposure of $\alpha1$ (BH4) facilitates interactions with other partners ● Redistribution between mitochondrial surface and cytosol in response to changes in $\Delta\psi$ or ΔpH, and protein–protein interactions
Bcl-2/Apaf-1-(ADP)	● Mitochondrial localization ● Anchoring of the zymogen caspase in the ternary complex ● Chaperonin-like function
Apaf-1-(ATP)/procaspase-3	● Nucleotide exchange (ADP/ATP) by chaperone Apaf-1-(ATP) releases the heterodimer Apaf-1-procaspase from ternary complex with Bcl-2 ● Hydrolysis of ATP allows processing of zymogen to active caspase-3 ● Dissociation of Apaf-1-(ADP) from the heterodimer releases active caspase-3
Apaf-1-(ADP)/Bcl-2	● Apaf-1-(ADP) reassociates with Bcl-2 and relocates to mitochondria ● Heterodimer Apaf-1-(ADP)/Bcl-2 associates with procaspase into the ternary complex ● Cycle may be completed

tarity is then the basis of specificity and would therefore determine the inclusion or exclusion of partners in the heterodimer, as well as the stability of the dimer. This is the basis for the model here presented.

5. Bcl-2 family members of death promoters and suppressors are channel proteins: counterintuitive or clue to mechanism?

The structure of Bcl-X_L provided a result of great impact towards the elucidation of the function of Bcl-2 family proteins. The 3-D structure displays a remarkable similarity to the pore forming domains of diphtheria toxin (DT-fragment B) [60] and colicins [61]. This similarity prompted several groups to examine the channel-forming activity of Bcl-2 family proteins, and to investigate the transport through the putative channels of elusive components of the death cascade. Biophysical analysis of recombinant Bcl-2 [62], Bcl-X_L [63] and Bax [64,65], all devoid of the C-terminal transmembrane anchor, showed that they indeed form ion channels after reconstitution in lipid bilayers. The new findings have been recently reviewed elsewhere [66], and here we focus on the properties that may be relevant to their mode of action in the death cascade.

The channel properties of each of these three proteins differ from one another in terms of single channel conductance, selectivity, and voltage dependence. However, they do show similarity with respect to DT and colicins in so far as acid pH and negatively charged lipids promote protein insertion and channel formation [67,68]. From a mechanistic point of view, all three Bcl-2 proteins form channels of heterogeneous conductances, consistent with the formation of conductive oligomers with different stoichiometries, and with the known propensity of these proteins to form homodimers and heterodimers [49,50,54,55]. A closer inspection of the 3-D structure indicates that helices α5 and α6 are the likely candidates to insert into the membrane. If a channel were formed by a Bcl-2 homodimer and each subunit were to contribute a helical pair of α5 and α6, it follows that a four helix bundle would be the structure underlying the observed channel activity. In line with this reasoning is the fact that a synthetic channel protein designed to adopt a four helix bundle topology displays a conductance of 20 pS, similar to the most frequent conductance measured for Bcl-2 [69]. It is, therefore, reassuring that a mutant Bcl-2 protein in which these two helices were deleted is devoid of channel activity [62]. In this context, sequence analysis shows that the signature sequence for the Bcl-2 family of apoptosis regulators is the amino-terminal half of α5 (consensus NWGRIVALFAF) in which A is an identical glutamate in human, rodent and murine Bcl-2 proteins, and tyrosine in the corresponding Bax proteins. The charged residues, R and E in Bcl-2 or R and Y in Bax, are separated by seven residues, two turns of helix apart. This locates them at the same face of the helix, presumably exposed to the channel lumen and in a key position to determine aspects of ionic selectivity [62,69]. The strong conservation of this signature sequence and its correspondence to a functional module of this family of proteins suggests that channel activity may be a clue to their cellular function(s).

It appears as counterintuitive to consider a channel forming protein localized to mitochondrial membranes as a death suppressor. After all, gramicidin, one of the best studied channel-forming peptides, is an uncoupler of oxidative phosphorylation [70]. It is more congruent to think of a death promoter as a channel former because a plethora of reports indicate that cell death is readily inducible by forming pores in cell membranes, from bacteria to eukaryotes [71]. This discrepancy may be resolved by considering the fact that pro- and anti-apoptotic proteins heterodimerize and that elimination of the death-promoter by the death-suppressor through dimerization may be at the core of this regulatory process [49,50,54,55]. Furthermore, if $\Delta\psi$ were involved in determining the activity of the heterodimers, as surmised from the voltage dependence for channel formation by Bcl-2 family proteins, then a plausible sequence of events that connect the mitochondria with these proteins may be envisioned: changes in $\Delta\psi$ would promote the association of Bcl-2 and Bax and restrict them to the mitochondrial surface. Dimer formation would promote dissociation of other entities of the death cascade from Bcl-2 and allow them to diffuse into the cytosol where these could interact with downstream components of the cascade. CsA, acting via a mitochondrial cyclophilin, may function as neuroprotectant

[20,21,25] by changing the protein–protein interacting surfaces of the partners. This model connects a mitochondrial membrane transport and anchoring activity with the regulation of the death cascade. Is there any evidence for such a scheme?

Analysis of gene products involved in the death cascade of the nematode *Caenorhabditis elegans* (cell death or CED genes) disclosed three classes of proteins involved in the process: CED-9, CED-4 and CED-3 (for review see [72,73]). The corresponding mammalian homologs have been identified and they are hereafter denoted as Bcl-2 family, Apaf-family (for apoptosis protease activating factors) and Caspase family (for cysteine-dependent, aspartate-specific protease), respectively (for review see [74]). The three families could be visualized as three independent, yet interacting modules and denoted as B, A, and C for Bcl-2, Apaf and Caspase families, respectively (Fig. 2). Numerous members for each of these sets of proteins have been identified and it is likely that more will emerge. The interactions between these proteins are beginning to be delineated and, therefore, a minimum sequence of events that may lead to cell death may be heuristically formulated. For simplicity, two states are described, a quiescent state associated with 'life' and the final state of the pathway-'death', and only a single prototype member of each family is selected. The model considers that the ternary complex BAC is stable in the quiescent state and that heterodimers are the functional information exchangers. Some specific features of the individual protein products provide constraints to the model and these are listed in the form of 'key heterodimers' in Table 1.

The starting point of the model is the quiescent state in which the ternary complex is stabilized by the ADP-form of Apaf-1 (A) [75–79] and is anchored via the C-terminal transmembrane domain of Bcl-2 (B2) to the mitochondrial outer membrane at the so-called zone of adhesion or ZOA, where the outer and inner mitochondrial membranes make contact [46–48]. In such state, the zymogen caspase (C) would be inactive.

5.1. Events at the mitochondrial surface

The signal cascade considers that a death stimulus will funnel into a change of mitochondrial $\Delta\psi$. Specifically, for excitotoxicity this could arise from glutamate-induced elevation of $[Ca^{2+}]_i$, production of free oxygen radicals and NO, ATP depletion or combinations thereof. All these events may lead the opening of the PTP. Attenuation of $\Delta\psi$ would promote insertion of Bax (BX, Fig. 2) into the mitochondrial membrane which would further collapse $\Delta\psi$; this is consistent with the voltage dependence for channel formation by Bax in lipid bilayers [64,65], and with the redistribution of cytosolic Bax to mitochondria during apoptosis [48]. This, in turn, may induce mitochondrial swelling and consequent release of cytochrome *c* [80–82] (also denoted as Apaf-2), other mitochondrial matrix enzymes [83] and ATP which may be required for caspase activation [75–84]. That the Bax channel provides the conduit through which cytochrome *c* is released from the intermembrane space to the cytosol has been considered [51,85], and that Bcl-X_L inhibits the collapse of $\Delta\psi$ and the release of cytochrome *c* has been reported [86]. Perhaps, the protective actions of Bcl-X_L [86] and Bcl-2 [80–82] arise from heterodimerization with Bax. The presumably higher affinity of Bcl-2 for Bax mediated by the α2 helix may promote heterodimerization with Bax (B2/BX, Fig. 2). Indeed, peptides with sequences patterned after the BH3 region of Bak and Bax and corresponding to α2 of Bcl-X_L are inhibitors of homodimer and heterodimer formation by Bcl-2 family members [87]. The propensity to form the Bcl-2/Bax binary complex, assisted by the released ATP which may facilitate ADP for ATP nucleotide exchange at the Apaf-1, may induce dissociation of the Apaf-1-(ATP)/zymogen complex (AC, Fig. 2).

5.2. Events in the cytosol

The released Apaf-1-(ATP)/zymogen heterodimer with the chaperone in the ATP form (AC-ATP) may allow processing of the zymogen to yield the active caspase [75–79]. This processing reaction may require ATP hydrolysis which may change the conformation of Apaf-1-(ADP) and facilitate its dissociation from the binary complex. Cytochrome *c* released from swollen mitochondria is also an activator of caspase 3 (CPP32) [77,80–82]. Apaf-1-(ADP) monomer may associate to Bcl-2 and free zymogen caspase to regenerate the heterotrimer and

thereby complete the cycle. Moreover, it was recently revealed that Bcl-2 is a caspase 3 (CED-3) protease inhibitor [88], thereby introducing another unsuspected role for monomeric Bcl-2 in the cascade, and that monomeric Bax in non-apoptotic cells is a soluble protein diffusely distributed throughout the cytosol [48]. Furthermore, Apaf-3 is caspase-9 which requires cytochrome *c* and ATP to become active [84].

The fundamental principle invoked here is that through a concerted set of protein–protein interactions, information is exchanged by specific heterodimers, one of the partners acting as a toxic protein and the second as its antidote. For the Bax/Bcl-2 heterodimer, Bax is known to collapse $\Delta\psi$ and perhaps provide the conduit for cytochrome *c* to exit from mitochondria into cytosol where it activates caspase 3; Bcl-2 allows recovery of $\Delta\psi$, inhibits release of caspase activating factors, including cytochrome *c* and prevents cell death. The presumed chaperonin activity of Apaf-1 allows it to act as an adaptor protein: in its ADP-form, it mediates the stability of ternary complex with Bcl-2 and the zymogen caspase; in its ATP form, it allows both the dissociation from Bcl-2 and the processing of the zymogen into an active caspase, implicated in the ultimate demise of the cell.

The physiological role of the B, A and C modules in vivo may be inferred from studies with transgenic mice, either by overexpression of the gene product or by gene ablation. For the B module, overexpression studies have clearly shown that Bcl-2 and Bcl-X_L act as neuronal survival factors in developmental paradigms, growth factor deprivation or ischemic insults (for review see [50,89–91]). Mice deficient in Bcl-2 do not show gross brain changes presumably resulting from functional redundancy, yet cerebellar granule neurons exhibit increased suceptibility to cell death [92], and motor and sensory neurons to degeneration during development [44]. Bax-deficient mice, however, show increase in neuron number in the superior cervical ganglia and increased protection of sympathetic neurons from cell death induced by nerve growth factor-withdrawal [45]. No information is yet available from gene ablation studies of the Apaf family. For the C module, mice deficient in the CED-3 related protease CPP32 show drastic alterations in brain development and markedly attenuated apoptosis in brain regions associated with major morphogenetic change during normal development [93]. Further, glutamate-induced apoptosis of cerebellar granule neurons involves activation of caspase-3 protease [94].

5.3. Modulation by protein phosphorylation

Phosphorylation on serine/threonine of Bcl-2 attenuates its anti-apoptotic activity whereas phosphorylation of Bad blocks its pro-apopoptotic activity, presumably by hindering dimerization. The Raf-1 kinase [95] as well as calcineurin, a phosphatase [96], bind to Bcl-2 via the α1 helix and get translocated from the cytosol to the mitochondrial surface. Heterodimerization of Bcl-2 with Bax suppresses complex formation with calcineurin [96]. Phosphorylation of the pro-apoptotic protein Bad by the serine/threonine protein kinase Akt [97] and by Raf-1 [95] releases it from Bcl-X_L at the mitochondrial surface and allows association of Bad with 14-3-3 restricting it to the cytosol. Dissociation of Bcl-X_L presumably allows it to resume its antiapoptotic activity via its interactions with either the downstream components of the caspase cascade, or its partners by relocating at the mitochondrial membrane. Noteworthy, Akt-mediated phosphorylation of Bad suppresses Bad-induced cell death in cerebellar granule neurons [97,98], and Bcl-2 blocks calcium-activated cell death produced by overexpression of calcineurin [99]. The selective recognition of kinases and phosphatases by the same domain of Bcl-2 (α1 or BH4), which is absent in many of the death promoters, implies that the resulting interactions may be essential for the regulation of cell survival. Notably, removal of α1 from Bcl-2 transforms it from a death suppressor to a death promoter [59].

6. Toxin-antidote bimodular design for the control of cell destruction

The notion that a programmed death cascade constitutes a selective mechanism to avoid random self-destruction [100,101] implies that the fundamental design of the molecular machinery destined for this function may be conserved in evolution. Apoptosis has been identified among several unicellular eukar-

yotes, remarkably the earliest mitochondrial eukaryotes such as *Trypanosoma cruzi* [102], *Trypanosoma brucei rhodensiense* [103], *Tetrahymena thermophila* [104], and *Dicyostellium discoideum* [105] (for review see [100]).

The occurrence of programmed self-destruction in prokaryotes has been inferred from considerations that moribund or starving populations undergo bacteriolysis as an 'altruistic' means to reduce competition for nutrients or to provide cellular contents for the support of the surviving cells [100]. Plasmid addiction modules, consisting of a toxin/antidote gene pair, are implicated in the programmed death of starving bacteria [101]. From this viewpoint, self-destruction is tightly coupled to survival and if the ratio of toxin to antidote is overpowering, death ensues. Plasmid addiction modules may encode restriction endonucleases and their cognate methylases, in which the latter (as antidote) modifies DNA to prevent the action of the restriction enzyme (the toxin). The diversity of the toxins and antidotes is broad. In the context of this review, it is pertinent to emphasize that the toxins include members of the colicin E1 family which are bacteriolytic by forming channels in target cells. *gef* and *hok* (for host killing) are intracellular toxins which are cytolytic by dissipating the membrane potential, presumably through the formation of ionic channels [106,107]. The antidote in this pair, e.g. *sok* (for suppression of killing) consists of antisense RNA bound to a precursor of the mRNA that encodes the toxin and thereby blocks its synthesis [108,109]. In *Escherichia coli*, the entericidin locus encodes two lipoproteins that may constitute another example of toxin/antidote gene pair: entericidin A is an antidote to the bacteriolytic entericidin B (Bishop, Leskiw, Hodges, Kay and Weiner, The entericidin locus of *E. coli* and its implications for programmed bacterial cell death, J. Mol. Biol. (1998) in press). Both proteins contain a lipoprotein processing site that would yield peptides of 23 and 26 amino acids, respectively. Synthetic peptides patterned after the corresponding sequences form amphipathic α-helices and exhibit a high propensity to form coiled-coils. As the entericidin AB pair, the coiled-coil of two amphipathic helices would provide a stable, yet inert entity. Individually, enterocidin B exhibits the hallmark characteristics of a channel-forming peptide that would insert into membranes and self-assemble into conductive oligomers [69]. In contrast, entericidin A, has features that would constrain it at the membrane interface restricting its insertion into the bilayer core [110]. Then, again, the AB pair may represent an efficacious design to suppress or unleash the channel activity of entericidin B and, therefore, control cell death.

A fascinating example of the toxin/antidote bimodular strategy adapted for the control of survival or death may be found in the family of peptides called defensins [111]. Defensins are small (∼30-mer) cationic peptides which exhibit cytotoxic and antimicrobial activity. The 3-dimensional structure of several members of the family has been solved by X-ray crystallography and NMR spectroscopy [112]. The fold of the monomer is conserved and consists of a triple stranded antiparallel β-sheet with a framework of six disulfide-linked cysteines. The cytolytic entity is presumably an oligomer of six dimers organized around a central aqueous pore [111]. It is surmised that a first step in killing is the binding of cationic defensins to acidic phosphatidylserine in the inner leaflet of the membrane bilayer; this is followed by a voltage-dependent insertion into the bilayer core and assembly of the transmembrane pore [111,112]. Human defensins, also known as human neutrophil peptides (HNP1–3), are synthesized as a 94 amino acid proprecursor which is post-translationally proteolyzed to inactive proHNP(20–94) [113]. This precursor consists of an N-terminal segment called the 'anionic propiece' which, in turn, is cleaved off to release the mature defensin HNP-1 and HNP-3(65–94) or HNP-2(66–94). In neutrophils, the mature defensins are stored in secretory granules. Remarkably, both modules are amphiphilic, and the 'anionic propiece' is an intramolecular inhibitor of the 'cationic defensin' cytotoxic activity [113]. Again, this suggests the occurrence of a toxin/antidote pair. The anionic propiece may act not only as an inhibitor, but also function as structural stabilizer – a chaperonin-like activity, or assist in membrane targeting. In other cellular systems or contexts, the two modules may act as independent molecules, as inferred from the occurrence of insect defensins, ∼4 kDa peptides from *Drosophila* [114] and scorpion [115]. Insect defensins conspicuously kill *Micrococcus luteus* by forming voltage-dependent pores which collapse the cell membrane potential [116].

Is it then conceivable that Bcl-2 proteins also constitute a toxin/antidote pair with the inherent combinatorial diversity that may arise from the plethora of family members operating as death promoters (toxin) or suppressors (antidote)?

7. Concluding remarks

An attempt was made to integrate evidence implicating mitochondrial channels in the death cascade. A plausible connection posits the notion that death signals (e.g. glutamate-induced Ca^{2+} overload in neurotoxicity) funneled via changes in mitochondrial $\Delta\psi$ (Fig. 1) selectively dysregulate a 'toxin–antidote' pair formed by death promoter/death suppressor members of the Bcl-2 family (Fig. 2). This in turn, would unleash an avalanche of events that lead to the imminent demise of the cell. Until more is known about the components of the cascade and their interactions than is known at present, it will be difficult to discern the key controlling steps in the network. With access to the wealth of sequence information from complete genomes of archaea (*M. jannaschii*), eubacteria (*E. coli*) as well eukaryotic (*Saccharomyces cerevisiae*) organisms (cf. [117]), the prospect of deciphering the connectivity between the components of the death cascade and understanding this fundamental process is an exciting challenge.

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References

[1] P. Nicotera, M. Ankarcrona, E. Bonfoco, S. Orrenius, S.A. Lipton, Neuronal necrosis and apoptosis: two distinct events induced by exposure to glutamate or oxidative stress, Adv. Neurol. 72 (1997) 95–101.

[2] D.W. Choi, Ischemia-induced neuronal apoptosis, Curr. Opin. Neurobiol. 6 (1996) 667–672.

[3] M.F. Beal, Mitochondria, free radicals, and neurodegeneration, Curr. Opin. Neurobiol. 6 (1996) 661–666.

[4] N.A. Simonian, J.T. Coyle, Oxidative stress in neurodegenerative diseases, Annu. Rev. Pharmacol. Toxicol. 36 (1996) 83–106.

[5] S.M. Rothman, J.W. Olney, Excitotoxicity and the NMDA receptor – still lethal after eight years, Trends Neurosci. 18 (1995) 57–58.

[6] J.A. Dani, M.L. Mayer, Structure and function of glutamate and nicotinic acetylcholine receptors, Curr. Opin. Neurobiol. 5 (1995) 310–317.

[7] S. Nakanishi, Y. Nakajima, A. Nomura, M. Masu, H. Iwakabe, Y. Hayashi, M. Yokoi, Functions and roles of glutamate receptors in synaptic transmission and plasticity, Cold Spring Harbor Symp. Quant. Biol. 61 (1996) 67–75.

[8] W.J. Koroshetz, M.A. Moskowitz, Emerging treatments for stroke in humans, Trends Pharmacol. Sci. 17 (1996) 227–339.

[9] V.P. Skulachev, Role of uncoupled and non-coupled oxidations in maintenance of safely low levels of oxygen and its one-electron reductants, Q. Rev. Biophys. 29 (1996) 169–202.

[10] T.E. Gunter, D.R. Pfeiffer, Mechanisms by which mitochondria transport calcium, Am. J. Physiol. 258 (1990) C755–786.

[11] Y. Tang, R.S. Zucker, Mitochondrial involvement in post-tetanic potentiation of synaptic transmission, Neuron 18 (1997) 483–491.

[12] F. Ichas, L.S. Jouaville, J.P. Mazat, Mitochondria are excitable organelles capable of generating and conveying electrical and calcium signals, Cell 89 (1997) 1145–1153.

[13] L.S. Jouaville, F. Ichas, E.L. Holmuhamedov, P. Camacho, J.D. Lechleiter, Synchronization of calcium waves by mitochondrial substrates in Xenopus laevis oocytes, Nature 377 (1995) 438–441.

[14] P. Bernardi, K.M. Broekemeier, D.R. Pfeiffer, Recent progress on regulation of the mitochondrial permeability transition pore; a cyclosporin-sensitive pore in the inner mitochondrial membrane, J. Bioenerg. Biomembr. 26 (1994) 509–517.

[15] S.L. Budd, D.G. Nicholls, Mitochondria, calcium regulation, and acute glutamate excitotoxicity in cultured cerebellar granule cells, J. Neurochem. 67 (1996) 2282–2291.

[16] J.T. Coyle, P. Puttfarcken, Oxidative stress, glutamate, and neurodegenerative disorders, Science 262 (1993) 689–695.

[17] B. Chance, Non-invasive approaches to tissue bioenergetics, Biochem. Soc. Trans. 22 (1994) 983–987.

[18] L.L. Dugan, S.L. Sensi, L.M. Canzoniero, S.D. Handran, S.M. Rothman, T.S. Lin, M.P. Goldberg, D.W. Choi, Mitochondrial production of reactive oxygen species in cortical neurons following exposure to N-methyl-D-aspartate, J. Neurosci. 15 (1995) 6377–6388.

[19] G.J. Wang, S.A. Thayer, Sequestration of glutamate-induced Ca^{2+} loads by mitochondria in cultured rat hippocampal neurons, J. Neurophysiol. 76 (1996) 1611–1621.

[20] R.J. White, I.J. Reynolds, Mitochondrial depolarization in glutamate-stimulated neurons: an early signal specific to excitotoxin exposure, J. Neurosci. 16 (1996) 5688–5697.
[21] A.F. Schinder, E.C. Olson, N.C. Spitzer, M. Montal, Mitochondrial dysfunction is a primary event in glutamate neurotoxicity, J. Neurosci. 16 (1996) 6125–6133.
[22] S.L. Budd, D.G. Nicholls, A reevaluation of the role of mitochondria in neuronal Ca^{2+} homeostasis, J. Neurochem. 66 (1996) 403–411.
[23] B. Khodorov, V. Pinelis, O. Vergun, T. Storozhevykh, N. Vinskaya, Mitochondrial deenergization underlies neuronal calcium overload following a prolonged glutamate challenge, FEBS Lett. 397 (1996) 230–234.
[24] N.K. Isaev, D.B. Zorov, E.V. Stelmashook, R.E. Uzbekov, M.B. Kozhemyakin, I.V. Victorov, Neurotoxic glutamate treatment of cultured cerebellar granule cells induces Ca^{2+}-dependent collapse of mitochondrial membrane potential and ultrastructural alterations of mitochondria, FEBS Lett. 392 (1996) 143–147.
[25] M. Ankarcrona, J.M. Dypbukt, S. Orrenius, P. Nicotera, Calcineurin and mitochondrial function in glutamate-induced neuronal cell death, FEBS Lett. 394 (1996) 321–324.
[26] M. Ankarcrona, J.M. Dypbukt, E. Bonfoco, B. Zhivotovsky, S. Orrenius, S.A. Lipton, P. Nicotera, Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function, Neuron 15 (1995) 961–973.
[27] S. Eimerl, M. Schramm, The quantity of calcium that appears to induce neuronal death, J. Neurochem. 62 (1994) 1223–1226.
[28] J.M. Dubinsky, S.M. Rothman, Intracellular calcium concentrations during 'chemical hypoxia' and excitotoxic neuronal injury, J. Neurosci. 11 (1991) 2545–2551.
[29] I. Marey-Semper, M. Gelman, M. Levi-Strauss, A selective toxicity toward cultured mesencephalic dopaminergic neurons is induced by the synergistic effects of energetic metabolism impairment and NMDA receptor activation, J. Neurosci. 15 (1995) 5912–5918.
[30] M.S. Saporito, R.E. Heikkila, S.K. Youngster, W.J. Nicklas, H.M. Geller, Dopaminergic neurotoxicity of 1-methyl-4-phenylpyridinium analogs in cultured neurons, relationship to the dopamine uptake system and inhibition of mitochondrial respiration, J. Pharmacol. Exp. Ther. 260 (1992) 1400–1409.
[31] G. Kroemer, The proto-oncogene Bcl-2 and its role in regulating apoptosis, Nature Med. 3 (1997) 614–620.
[32] T. Hirsch, I. Marzo, G. Kroemer, Role of the mitochondrial permeability transition pore in apoptosis, Biosci. Rep. 17 (1997) 67–76.
[33] P. Bernardi, V. Petronilli, The permeability transition pore as a mitochondrial calcium release channel: a critical appraisal, J. Bioenerg. Biomembr. 28 (1996) 131–138.
[34] V.P. Skulachev, Why are mitochondria involved in apoptosis? Permeability transition pores and apoptosis as selective mechanisms to eliminate superoxide-producing mitochondria and cell, FEBS Lett. 397 (1996) 7–10.
[35] K. Hyrc, S.D. Handran, S.M. Rothman, M.P. Goldberg, Ionized intracellular calcium concentration predicts excitotoxic neuronal death: observations with low-affinity fluorescent calcium indicators, J. Neurosci. 17 (1997) 6669–6677.
[36] B.S. Kristal, J.M. Dubinsky, Mitochondrial permeability transition in the central nervous system: induction by calcium cycling-dependent and -independent pathways, J. Neurochem. 69 (1997) 524–538.
[37] V. Petronilli, C. Cola, S. Massari, R. Colonna, P. Bernardi, Physiological effectors modify voltage sensing by the cyclosporin A-sensitive permeability transition pore of mitochondria, J. Biol. Chem. 268 (1993) 21939–21945.
[38] S. Hortelano, B. Dallaporta, N. Zamzami, T. Hirsch, S.A. Susin, I. Marzo, L. Bosca, G. Kroemer, Nitric oxide induces apoptosis via triggering mitochondrial permeability transition, FEBS Lett. 410 (1997) 373–377.
[39] M. Leist, E. Fava, C. Montecucco, P. Nicotera, Peroxynitrite and nitric oxide donors induce neuronal apoptosis by eliciting autocrine excitotoxicity, Eur. J. Neurosci. 9 (1997) 1488–1498.
[40] R.M. Kramer, J.D. Sharp, Structure, function and regulation of Ca^{2+}-sensitive cytosolic phospholipase A2 (cPLA2), FEBS Lett. 410 (1997) 49–53.
[41] J.V. Bonventre, Z. Huang, M.R. Taheri, E. O'Leary, E. Li, M.A. Moskowitz, A. Sapirstein, Reduced fertility and postischemic brain injury in mice deficient in cytosolic phospholipase A2, Nature 390 (1997) 622–625.
[42] A. Srinivasan, L.M. Foster, M.P. Testa, T. Ord, R.W. Keane, D.E. Bredesen, C. Kayalar, Bcl-2 expression in neural cells blocks activation of ICE/CED-3 family proteases during apoptosis, J. Neurosci. 16 (1996) 5654–5660.
[43] A.N. Murphy, D.E. Bredesen, G. Cortopassi, E. Wang, G. Fiskum, Bcl-2 potentiates the maximal calcium uptake capacity of neural cell mitochondria, Proc. Natl. Acad. Sci. USA 93 (1996) 9893–9898.
[44] T.M. Michaelidis, M. Sendtner, J.D. Cooper, M.S. Airaksinen, B. Holtmann, M. Meyer, H. Thoenen, Inactivation of bcl-2 results in progressive degeneration of motoneurons, sympathetic and sensory neurons during early postnatal development, Neuron 17 (1996) 75–89.
[45] T.L. Deckwerth, J.L. Elliott, C.M. Knudson, E.M. Johnson Jr., W.D. Snider, S.J. Korsmeyer, BAX is required for neuronal death after trophic factor deprivation and during development, Neuron 17 (1996) 401–411.
[46] S. Krajewski, S. Tanaka, S. Takayama, M.J. Schibler, W. Fenton, J.C. Reed, Investigation of the subcellular distribution of the bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes, Cancer Res. 53 (1993) 4701–4714.
[47] Y.T. Hsu, K.G. Wolter, R.J. Youle, Cytosol-to-membrane redistribution of Bax and Bcl-X_L during apoptosis, Proc. Natl. Acad. Sci. USA 94 (1997) 3668–3672.
[48] K.G. Wolter, Y.T. Hsu, C.L. Smith, A. Nechushtan, X.-G. Xi, R.J. Youle, Movement of Bax from cytosol to mitochondria during apoptosis, J. Cell Biol. 139 (1997) 1281–1292.

[49] C.M. Knudson, S.J. Korsmeyer, Bcl-2 and Bax function independently to regulate cell death, Nature Genet. 16 (1997) 358–363.
[50] D.E. Merry, S.J. Korsmeyer, Bcl-2 gene family in the nervous system, Annu. Rev. Neurosci. 20 (1997) 245–267.
[51] E. Yang, S.J. Korsmeyer, Molecular thanatopsis: a discourse on the BCL2 family and cell death, Blood 88 (1996) 386–401.
[52] J.C. Reed, Double identity for proteins of the Bcl-2 family, Nature 387 (1997) 773–776.
[53] M.E. Peter, A.E. Heufelder, M.O. Hengartner, Advances in apoptosis research, Proc. Natl. Acad. Sci USA 94 (1997) 12736–12737.
[54] T.W. Sedlak, Z.N. Oltvai, E. Yang, K. Wang, L.H. Boise, C.B. Thompson, S.J. Korsmeyer, Multiple Bcl-2 family members demonstrate selective dimerizations with Bax, Proc. Natl. Acad. Sci. USA 92 (1995) 7834–7838.
[55] Z.N. Oltvai, C.L. Milliman, S.J. Korsmeyer, Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death, Cell 74 (1993) 609–619.
[56] S.W. Muchmore, M. Sattler, H. Liang, R.P. Meadows, J.E. Harlan, H.S. Yoon, D. Nettesheim, B.S. Chang, C.B. Thompson, S.L. Wong et al., X-ray and NMR structure of human Bcl-X_L, an inhibitor of programmed cell death, Nature 381 (1996) 335–341.
[57] M. Sattler, H. Liang, D. Nettesheim, R.P. Meadows, J.E. Harlan, M. Eberstadt, H.S. Yoon, S.B. Shuker, B.S. Chang, A.J. Minn et al., Structure of Bcl-X_L-Bak peptide complex: recognition between regulators of apoptosis, Science 275 (1997) 983–986.
[58] J. Zha, H. Harada, K. Osipov, J. Jockel, G. Waksman, S.J. Korsmeyer, BH3 domain of BAD is required for heterodimerization with BCL-XL and pro-apoptotic activity, J. Biol. Chem. 272 (1997) 24101–24104.
[59] E.H.-Y. Cheng, D.G. Kirsch, R.J. Clem, R. Ravi, M.B. Kastan, A. Bedi, K. Ueno, J.M. Hardwick, Conversion of Bcl-2 to a Bax-like death effector by caspases, Science 278 (1997) 1966–1968.
[60] S. Choe, M.J. Bennett, G. Fujii, P.M.G. Curmi, K.A. Kantardjieff, R.J.E. Lutz, R.J. Collier, D. Eisenberg, Crystal structure of diphtheria toxin, Nature 357 (1992) 216–222.
[61] M. Wiener, D. Freymann, P. Ghosh, R.M. Stroud, Crystal structure of colicin Ia, Nature 385 (1997) 461–464.
[62] S.L. Schendel, Z. Xie, M.O. Montal, S. Matsuyama, M. Montal, J.C. Reed, Channel formation by antiapoptotic protein Bcl-2, Proc. Natl. Acad. Sci. USA 94 (1997) 5113–5118.
[63] A.J. Minn, P. Velez, S.L. Schendel, H. Liang, S.W. Muchmore, S.W. Fesik, M. Fill, C.B. Thompson, Bcl-X_L forms an ion channel in synthetic lipid membranes, Nature 385 (1997) 353–357.
[64] B. Antonsson, F. Conti, A. Ciavatta, S. Montessuit, S. Lewis, I. Martinou, L. Bernasconi, A. Bernard, J.-J. Mermod, G. Mazzei, K. Maundrell, F. Gambale, R. Sadoul, J.-C. Martinou, Inhibition of Bax channel-forming activity by Bcl-2, Science 277 (1997) 370–372.
[65] P.H. Schlesinger, A. Gross, X.-M. Yin, K. Yamamoto, M. Saito, G. Waksman, S.J. Korsmeyer, Comparison of the ion channel characteristics of proapoptotic Bax and antiapoptotic Bcl-2, Proc. Natl. Acad. Sci. USA 94 (1997) 11357–11362.
[66] S. Schendel, M. Montal, J.C. Reed, Bcl-2 family proteins as ion channels, Cell Death Differ. 5 (1998) 372–380.
[67] P. Elkins, A. Bunker, W.A. Cramer, C.V. Stauffacher, A mechanism for toxin insertion into membranes is suggested by the crystal structure of the channel-forming domain of colicin E1, Structure 5 (1997) 443–458.
[68] J.J. Donovan, M.I. Simon, M. Montal, Insertion of diphtheria toxin into and across membranes: role of phosphoinositide asymmetry, Nature 298 (1982) 669–672.
[69] M. Montal, Design of molecular function: channels of communication, Annu. Rev. Biophys. Biomol. Struct. 24 (1995) 31–57.
[70] M. Montal, B. Chance, C.P. Lee, Uncoupling of submitochondrial particles by Gramicidin, FEBS Lett. 6 (1970) 209–212.
[71] B. Bechinger, Structure and functions of channel-forming peptides: magainins, cecropins, melittin and alamethicin, J. Membr. Biol. 156 (1997) 197–211.
[72] M.O. Hengartner, H.R. Horvitz, Programmed cell death in Caenorhabditis elegans, Curr. Opin. Genet. Dev. 4 (1994) 581–586.
[73] M.S. Spector, S. Desnoyers, D.J. Hoeppner, M.O. Hengartner, C. Interaction between the, elegans cell-death regulators CED-9 and CED-4, Nature 385 (1997) 653–656.
[74] M.D. Jacobson, Apoptosis: Bcl-2-related proteins get connected, Curr. Biol. 7 (1997) R277–R281.
[75] A.M. Chinnaiyan, D. Chaudhary, K. O'Rourke, E.V. Koonin, V.M. Dixit, Role of CED-4 in the activation of CED-3, Nature 388 (1997) 728–729.
[76] A.M. Chinnaiyan, K. O'Rourke, B.R. Lane, V.M. Dixit, Interaction of CED-4 with CED-3 and CED-9: a molecular framework for cell death, Science 275 (1997) 1122–1126.
[77] H. Zou, W.J. Henzel, X. Liu, A. Lutschg, X. Wang, Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3, Cell 90 (1997) 405–413.
[78] S. Seshagiri, L.K. Miller, Caenorhabditis elegans CED-4 stimulates CED-3 processing and CED-3-induced apoptosis, Curr. Biol. 7 (1997) 455–460.
[79] D. Wu, H.D. Wallen, G. Nunez, Interaction and regulation of subcellular localization of CED-4 by CED-9, Science 275 (1997) 1126–1129.
[80] R.M. Kluck, E. Bossy-Wetzel, D.R. Green, D.D. Newmeyer, The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis, Science 275 (1997) 1132–1136.
[81] J. Yang, X. Liu, K. Bhalla, C.N. Kim, A.M. Ibrado, J. Cai, T.I. Peng, D.P. Jones, X. Wang, Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked, Science 275 (1997) 1129–1132.
[82] X. Liu, C.N. Kim, J. Yang, R. Jemmerson, X. Wang, Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c, Cell 86 (1996) 147–157.

[83] S. Estrada-O, M. Montal, H. Célis, A. Cárabez, Energy-dependent control of the latency of mitochondrial enzymes, Eur. J. Biochem. 12 (1970) 227–235.
[84] P. Li, D. Nijhawan, I. Budihardjo, S.M. Srinivasula, M. Ahmad, E.S. Alnemri, X. Wang, Cytochrome c and dATP-dependent formation of Apaf-1/Caspase-9 complex initiates an apoptotic protease cascade, Cell 91 (1997) 479–489.
[85] S. Manon, B. Chaudhuri, M. Buerin, Release of cytochrome c and decrease of cytochrome c oxidase in Bax-expressing yeast cells, and prevention of these effects by coexpression of Bcl-XL, FEBS Lett. 415 (1997) 29–32.
[86] M.G. Vander Heiden, N.S. Chandel, E.K. Williamson, P.T. Schumacker, C.B. Thompson, Bcl-X_L regulates the membrane potential and volume homeostasis of mitochondria, Cell 91 (1997) 627–637.
[87] J.L. Diaz, T. Oltersdorf, W. Horne, M. McConnell, G. Wilson, S. Weeks, T. Garcia, L.C. Fritz, A common binding site mediates heterodimerization and homodimerization of Bcl-2 family members, J. Biol. Chem. 272 (1997) 11350–11355.
[88] D. Xue, H. Horvitz, *Caenorhabditis elegans* CED-9 protein is a bifunctional cell-death inhibitor, Nature 390 (1997) 305–308.
[89] J.C. Martinou, M. Dubois-Dauphin, J.K. Staple, I. Rodriguez, H. Frankowski, M. Missotten, P. Albertini, D. Talabot, S. Catsicas, C. Pietra et al., Overexpression of BCL-2 in transgenic mice protects neurons from naturally occurring cell death and experimental ischemia, Neuron 13 (1994) 1017–1030.
[90] D.F. Chen, G.E. Schneider, J.C. Martinou, S. Tonegawa, Bcl-2 promotes regeneration of severed axons in mammalian CNS, Nature 385 (1997) 434–439.
[91] J. Chen, J.G. Flannery, M.M. LaVail, R.H. Steinberg, J. Xu, M.I. Simon, Bcl-2 overexpression reduces apoptotic photoreceptor cell death in three different retinal degenerations, Proc. Natl. Acad. Sci. USA 93 (1996) 7042–7047.
[92] H. Tanabe, Y. Eguchi, S. Kamada, J.C. Martinou, Y. Tsujimoto, Susceptibility of cerebellar granule neurons derived from Bcl-2-deficient and transgenic mice to cell death, Eur. J. Neurosci. 9 (1997) 848–856.
[93] K. Kuida, T.S. Zheng, S. Na, C. Kuan, D. Yang, H. Karasuyama, P. Rakic, R.A. Flavell, Decreased apoptosis in the brain and premature lethality in CPP32-deficient mice, Nature 384 (1996) 368–372.
[94] Y. Du, K.R. Bales, R.C. Dodel, E. Hamilton-Byrd, J.W. Horn, D.L. Czilli, L.K. Simmons, B. Ni, S.M. Paul, Activation of a caspase 3-related cysteine protease is required for glutamate-mediated apoptosis of cultured cerebellar granule neurons, Proc. Natl. Acad. Sci USA 94 (1997) 11657–11662.
[95] H.G. Wang, U.R. Rapp, J.C. Reed, Bcl-2 targets the protein kinase Raf-1 to mitochondria, Cell 87 (1996) 629–638.
[96] F. Shibasaki, E. Kondo, T. Akagi, F. McKeon, Suppression of signalling through transcription factor NF-AT by interactions between calcineurin and Bcl-2, Nature 386 (1997) 728–731.
[97] S.R. Datta, H. Dudek, X. Tao, S. Masters, H. Fu, Y. Gotoh, M.E. Greenberg, Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery, Cell 91 (1997) 231–241.
[98] H. Dudek, S.R. Datta, T.F. Franke, M.J. Birnbaum, R. Yao, G.M. Cooper, R.A. Segal, D.R. Kaplan, M.E. Greenberg, Regulation of neuronal survival by the serine-threonine protein kinase Akt, Science 275 (1997) 661–665.
[99] F. Shibasaki, F. McKeon, Calcineurin functions in Ca(2+)-activated cell death in mammalian cells, J. Cell Biol. 131 (1995) 735–743.
[100] J.C. Ameisen, The origin of programmed cell death, Science 272 (1996) 1278–1279.
[101] M.B. Yarmolinsky, Programmed cell death in bacterial populations, Science 267 (1995) 836–837.
[102] J.C. Ameisen, T. Idziorek, O. Billaut-Multo, M. Loyens, J.-P. Tissier, A. Potentier, A. Ouaissi, Apoptosis in a unicellular eukaryote (Trypanosoma cruzi): implications for the evolutionary origin and role of programmed cell death in the control of cell proliferation and survival, Cell Death Differ. 2 (1996) 285–300.
[103] S.C. Welburn, C. Dale, D. Ellis, R. Beecroft, T.W. Pearson, Apoptosis in procyclic Trypanosoma brucei rhodesiense in vitro, Cell Death Differ. 3 (1996) 229–236.
[104] S.T. Christensh, D.N. Wheatley, M.I. Rasmussen, L. Rasmussen, Mechanisms controlling death, survival and proliferation in a model unicellular eukaryote Tetrahymena thermophila, Cell Death Differ. 2 (1995) 301–308.
[105] S. Cornillon, C. Foa, J. Davoust, N. Buonavista, J.D. Gross, P. Golstein, Programmed cell death in Dictyostelium, J. Cell Sci. 107 (1994) 2691–2704.
[106] S. Molin, L. Boe, L.B. Jensen, C.S. Kristensen, M. Givskov, J.L. Ramos, A.K. Bej, Suicidal genetic elements and their use in biological containment of bacteria, Annu. Rev. Microbiol. 47 (1993) 139–166.
[107] T. Thisted, A.K. Nielsen, K. Gerdes, Mechanism of post-segregational killing: translation of Hok, SrnB and Pnd mRNAs of plasmids R1, F and R483 is activated by 3′-end processing, EMBO J. 13 (1994) 1950–1959.
[108] T. Franch, K. Gerdes, Programmed cell death in bacteria: translational repression by mRNA end-pairing, Mol. Microbiol. 21 (1996) 1049–1060.
[109] R.B. Jensen, K. Gerdes, Programmed cell death in bacteria: proteic plasmid stabilization systems, Mol. Microbiol. 17 (1995) 205–210.
[110] B. Bechinger, L.M. Gierasch, M. Montal, M. Zasloff, S.J. Opella, Orientations of helical peptides in membrane bilayers by solid state NMR spectroscopy, Solid State Nucl. Magnet. Reson. 7 (1996) 185–191.
[111] S.C. White, W.C. Wimley, M.E. Selsted, Structure, function, and membrane integration of defensins, Curr. Opin. Struct. Biol. 4 (1995) 521–527.
[112] G.R. Zimmermann, P. Legault, M.E. Selsted, A. Pardi, Solution structure of bovine neutrophil beta-defensin-12: the peptide fold of the beta-defensins is identical to that

of the classical defensins, Biochemistry 34 (1995) 13663–13671.
[113] E.V. Valore, E. Martin, S.S. Harwig, T. Ganz, Intramolecular inhibition of human defensin HNP-1 by its propiece, J. Clin. Invest. 97 (1996) 1624–1629.
[114] J.L. Dimarcq, D. Hoffmann, M. Meister, P. Bulet, R. Lanot, J.M. Reichhart, J.A. Hoffmann, Characterization and transcriptional profiles of a Drosophila gene encoding an insect defensin. A study in insect immunity, Eur. J. Biochem 221 (1994) 201–209.
[115] B. Cornet, J.M. Bonmatin, C. Hetru, J.A. Hoffmann, M. Ptak, F. Vovelle, Refined three-dimensional solution structure of insect defensin A, Structure 3 (1995) 435–448.
[116] S. Cociancich, A. Ghazi, C. Hetru, J.A. Hoffmann, L. Letellier, Insect defensin, an inducible antibacterial peptide, forms voltage-dependent channels in Micrococcus luteus, J. Biol. Chem. 268 (1993) 19239–19245.
[117] R.L. Tatusov, E.V. Koonin, D.J. Lipman, A genomic perspective on protein families, Science 278 (1997) 631–637.