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Review

Adenine nucleotide translocase: a component of the phylogenetically conserved cell death machinery

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Lethal mitochondrial membrane permeabilization has been depicted as the result of two fundamentally distinct processes, namely primary mitochondrial outer membrane permeabilization (MOMP) *versus* permeability transition (PT) ignited at the level of the mitochondrial inner membrane. MOMP and PT have been connected to apoptosis and necrosis, respectively. Moreover, it has been thought that MOMP was mediated by pro-apoptotic multidomain proteins of the Bcl-2 family (Bax and Bak), which would operate near-to-independently from the permeability transition pore complex (PTPC) composed by voltage-dependent anion channel (VDAC), adenine nucleotide translocase (ANT) and cyclophilin D. A recent paper in *Molecular and Cellular Biology* now reveals the obligate contribution of one particular ANT isoform to the execution of developmental and homeostatic cell death in *Caenorhabditis elegans*. The physical and functional interaction between CED-9, the sole multidomain Bcl-2 protein of *C. elegans*, and ANT emphasizes the existence of an intricate, phylogenetically conserved crosstalk between Bcl-2 family proteins and constituents of the PTPC. In this issue of *Cell Death and Differentiation*, Malorni *et al.* further corroborate this notion by showing that type 2 transglutaminase (TG2) is essential for the correct assembly/function of ANT1, and that, at least in some experimental settings, TG2 might be required to enable and/or stabilize the pro-apoptotic association of Bax with ANT1.
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The 'core' machinery of apoptosis was discovered by screening *Caenorhabditis elegans* mutations that suppressed developmental cell death, yielding a number of genes whose mammalian equivalents also have an important function in physiological and disease-associated apoptosis. Once it has been transcriptionally upregulated, EGL-1 (the only BH3-only protein encoded by *C. elegans*) disrupts a molecular complex composed by CED-9 (a Bcl-2 ortholog localized in mitochondrial membranes) and CED-4 (the nematode counterpart of Apaf-1, which is also normally present at mitochondria). As a consequence, CED-4 becomes free to translocate to the nuclear envelope and to activate CED-3 (the worm caspase), thereby inducing apoptosis.[1,2] Now, a new protein, WAN-1, the nematode ortholog of mammalian adenine nucleotide translocase (ANT) has been added to the 'core' machinery.[3]

The 'Core' Machinery for Cell Death Execution

The scenario of a conserved cell death 'core' machinery composed by EGL-1, CED-9, CED-4 and CED-3 has been dominating the field of cell death research up to the point that some investigators suggested the existence of a ternary molecular complex made by Apaf-1, caspase-9 and the Bcl-2 homolog Bcl-X_L also in mammalian cells,[4,5] which turned out to be a bold artifact.[6–8] Similarly, the idea that EGL-1, CED-9, CED-4 and CED-3 might have other functions than mediating cell death (EGL-1 as an inducer of autophagy,[9] CED-4 as part of the intra-S-phase DNA damage checkpoint,[2] CED-3 in the innate immune system[10]) met incredulity and resistance. Prominent researchers, who had been educated in the paradigm of the 'core' machinery, virulently combated the idea that additional processes like mitochondrial outer membrane permeabilization (MOMP) might have a cardinal function in the regulation of cell death, relegating mitochondria—at best—to an auxiliary role within an 'amplification loop'.[11,12] Indeed, in contrast to Apaf-1, CED-4 lacks the C-terminal WD40 repeats that mediate the interaction between Apaf-1 and cytochrome *c*[13] and CED-4-mediated CED-3 activation does not require any additional proteins.[1] This difference between nematodes and mammals has stimulated an intense debate on the evolution of cell death. Some investigators suggested that the mitochondrial

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Abbreviations: AAC, ATP/ADP carrier; AIF, apoptosis-inducing factor; ANT, adenine nucleotide translocase; ANT2BP, ANT2-binding protein; BH3, Bcl-2 homology domain 3; $\Delta\psi_m$, mitochondrial transmembrane potential; DDG, deoxy-D-glucose; DRP-1, dynamin-related protein-1; MCP, mitochondrial carrier protein; mitoK_{ATP}, mitochondrial ATP-sensitive K^+ channel; MMP, mitochondrial membrane permeabilization; MOMP, mitochondrial outer membrane permeabilization; MTCH, mitochondrial carrier homolog; PCD, programmed cell death; PS, phosphatidylserine; PT, permeability transition; PTPC, permeability transition pore complex; ROS, reactive oxygen species; STS, staurosporine; TG2, type 2 transglutaminase; VDAC, voltage-dependent anion channel
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'amplification loop' has been added to the 'core' machinery during the evolution of higher animals.[11] However, another plausible scenario predicts that the link between mitochondrial stress, cytochrome *c* release and apoptosis induction that regulates intrinsic apoptosis in mammals (and presumably also in a variety of primitive animals including mollusks)[14] has been deleted during the evolution of nematodes (which at the adult stage are mainly composed of post-mitotic cells and hence cannot afford a stress-induced apoptotic default pathway).[15,16] Irrespective of these speculative considerations, it is clear that the pioneering work on *C. elegans* cell death has inspired the field of mammalian cell death research, especially during the 1980s and the 1990s.

Over the last decade, the wind has turned, and the discovery of proteins having an important function in human or murine cell death has inspired work in *C. elegans*. Thus, the nematode system was expected to confirm (or invalidate) the phylogenetic conservation—and hence the presumed physiological importance—of processes and molecules first recognized in the context of mammalian cell death. The discovery of a caspase-independent lethal pathway mediated by apoptosis-inducing factor (AIF) in mammals[17] triggered studies in *C. elegans*, which showed that during developmental cell death WAH-1 (the worm ortholog of AIF) translocates from the mitochondrial intermembrane space to the nucleus and that the knockout of *wah-1* led to the survival of ectopic cells, in particular in the context of moderate *ced-3* loss-of-function alleles.[18] WAH-1 turned out to collaborate with another mitochondrial factor, CPS-6 (the nematode ortholog of endonuclease G), which reportedly co-translocates with WAH-1 to the nucleus where the WAH-1-CPS-6 complex mediates DNA degradation,[18] as well as with the phospholipid scramblase SCRM-1, a plasma membrane protein that favors the exposure of phosphatidylserine (PS) on the surface of apoptotic cells.[19] These observations were in line with the facts that AIF can acquire a nuclease function in mammals and yeast cells (by interacting with proteins from the cyclophilin family),[20] and can stimulate PS exposure in murine cells[17] (Figure 1). These results indirectly revealed the importance of MOMP (which is required for the release of WAH-1/AIF and CPS-6/endonuclease G from the mitochondrial intermembrane space) in the developmental cell death program of *C. elegans*.

In mammalian cells undergoing mitochondrial membrane permeabilization (MMP), cytochrome *c* (as well as several other mitochondrial intermembrane space proteins including AIF and endonuclease G) is released into the cytosol, where it triggers the caspase cascade.[21] On the contrary, the worm ortholog of cytochrome *c* is retained in mitochondria, even in conditions that allow for the mitochondrio-cytosolic translocation of WAH-1 and CPS-6. As mentioned above, the activation of CED-3 by CED-4 occurs independent of cytochrome *c*[1] (Figure 2). Nonetheless, the mechanisms that account for the retention of cytochrome *c* in the mitochondria of dying

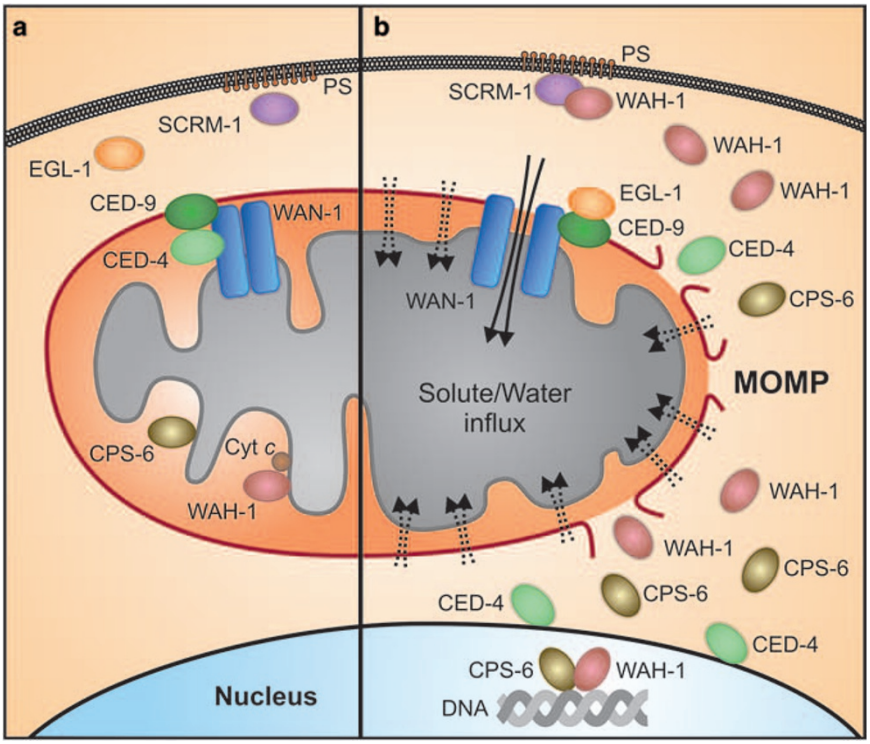

Figure 1 WAN-1 cooperates with the 'core' machinery to promote programmed cell death in *Caenorhabditis elegans*. (**a**) In physiological conditions, WAN-1 is embedded in the mitochondrial inner membrane, where it interacts with CED-9 and CED-4. (**b**) The cell death initiator EGL-1 can disrupt the ternary complex made of WAN-1, CED-9 and CED-4, leading to osmotic swelling of the mitochondrial matrix and eventually to mitochondrial outer membrane permeabilization (MOMP). This allows for the release into the cytosol of several mitochondrial proteins. Thus, CED-4 clusterizes in the perinuclear region, thereby facilitating CED-3 processing and cell death. WAH-1 not only co-translocates with CPS-6 to the nucleus to promote DNA degradation, but also interacts with SCRM-1 at the plasma membrane, thereby favoring the exposure of phosphatidylserine (PS) on the surface of dying cells

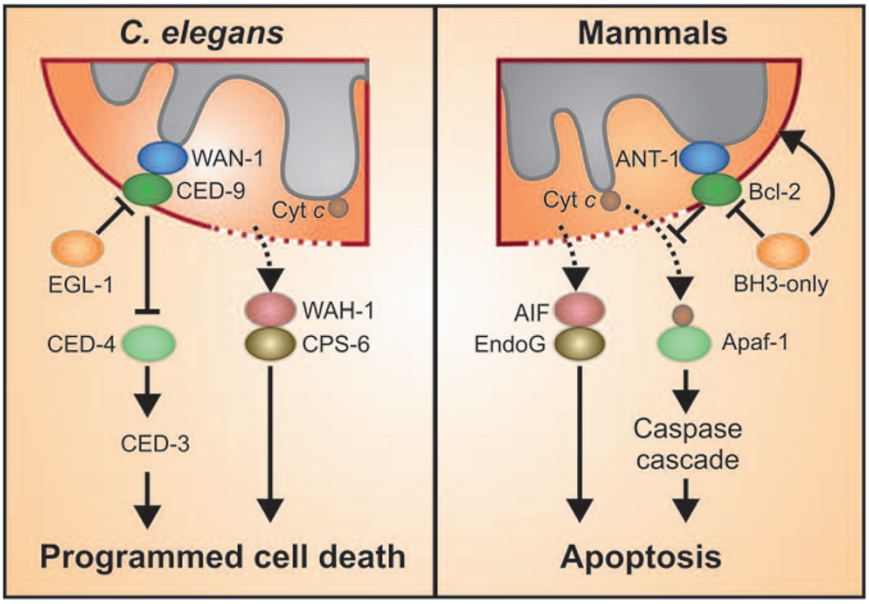

Figure 2 Conservation of the 'core' machinery for cell death across nematodes and mammals. The main processes and the key players that drive programmed cell death in *Caenorhabditis elegans* and apoptosis in mammalian cells display a high level of conservation throughout evolution. However, some important differences exist, such as the release of cytochrome *c* (Cyt *c*) from the mitochondrial intermembrane space, an event that seems to occur exclusively in mammalian cells. AIF, apoptosis-inducing factor; ANT-1, adenine nucleotide translocase 1; Apaf-1, apoptotic peptidase activating factor 1; BH3, Bcl-2 homology domain 3; EndoG, endonuclease G

C. elegans cells remain obscure. Reportedly, cytochrome *c* is anchored to mitochondria through both electrostatic and hydrophobic interactions with acidic phospholipids, and in particular with cardiolipin (which is found primarily in the mitochondrial inner membrane). In mammalian cells, during the early phase of apoptosis, excess reactive oxygen species (ROS) generated by mitochondria favor the oxidation of cardiolipin, thereby allowing for the detachment of cytochrome *c*. On MMP, soluble cytochrome *c* can then be released into the cytoplasm.[22,23] As in dying *C. elegans* cells, cytochrome *c* fails to translocate to the cytoplasm, it will be interesting to know whether programmed cell death (PCD) in nematodes is associated with ROS overproduction, or, alternatively, whether the worm antioxidant systems are sufficient to keep cardiolipin in a non-oxidized state that prevents cytochrome *c* detachment from the mitochondrial inner membrane.

An additional hint that mitochondria might contribute to cell death in *C. elegans* came from the observation that the transgene-enforced expression of a dominant-negative mutant of dynamin-related protein-1 (DRP-1) inhibited mitochondrial fragmentation associated PCD in *C. elegans*, while it caused a significant (though minor) inhibition of PCD.[24] These findings were apparently backed up by experiments in which CED-9 was ectopically overexpressed in mammalian cells and shown to promote EGL-1-inhibitable mitochondrial clustering.[25] However, as these experiments were based on the overexpression of xenogenous proteins, they may have yielded results of poor physiological significance. Moreover, recent studies performed in more physiological settings, failed to confirm any role for CED-9 or EGL-1 in the mitochondrial dynamics (fission and fusion) of *C. elegans*.[26,27] This represents an intensely debated topic, yet the overall tendency in the field is to admit the divorce between MOMP, which (at least in mammalian cells) is required for apoptosis, and mitochondrial fragmentation, which is dispensable for both MOMP and apoptosis.[28,29]

ANT: a Novel Ingredient of the 'Core' Machinery

A recent paper by Shen *et al*.[3] now reveals that the constitutive mitochondrial membrane protein ANT is part of the 'core' machinery for apoptosis in *C. elegans*. ANT (also called ATP/ADP carrier, AAC, in yeast) mediates the exchange (antiport) of ATP (which is synthesized in the mitochondrial matrix) and ADP (which derived from ATP consumption in the rest of the cell). The ANT dimer exists in two confirmations that are commonly referred to as the matrix (m) and the cytosolic (c) states, the latter being compatible with the formation of a high-conductance channel that can dissipate the mitochondrial transmembrane potential ($\Delta\psi_m$) and cause the colloidosmotic swelling of the mitochondrial matrix.[30] This phenomenon, which is the manifestation of an abrupt increase in the permeability to small solutes of the mitochondrial inner membrane, has been widely studied on isolated mitochondria and is known as 'permeability transition' (PT).[31] The implication of ANT in apoptosis was first suggested by pharmacological experiments showing that atractyloside (which favors the c-state of ANT) or its cell-permeable derivative carboxyatractyloside can induce apoptosis, whereas bongkrekate (which favors the m-state of ANT) can inhibit $\Delta\psi_m$ loss and prevent apoptosis. This was demonstrated in various experimental paradigms including intact cells (such as thymocytes exposed to glucocorticoids)[32,33] and cell-free systems (in which purified mitochondria were added to isolated nuclei and apoptosis was monitored by nuclear shrinkage).[34] Thus, bongkrekate has been instrumental for the first demonstration that apoptosis of mammalian cells is regulated by mitochondria.[32–34] However, both atractyloside and bongkrekate

lead to ATP depletion (because they inhibit the vital ATP/ADP antiporter function of ANT) and hence are highly toxic, a fact that limits their utility as investigational drugs. Bongkrekate (but not atractyloside) suppresses apoptosis of *C. elegans* germline cells,[3] yet is too toxic to be used for the long-term manipulation of developmental cell death. Therefore, subtler, genetic studies were required to investigate the contribution of ANT to cell death regulation in nematodes.

In *C. elegans*, the knockout of genes coding for three (out of four) worm ANT isoforms (WAN-2, WAN-3 and WAN-4) did not influence developmental and germline cell death.[3] Surprisingly, the *wan-1* knockout yielded *per se* a lethal phenotype,[3] which explains *a posteriori* why this protein has never been identified as a regulator of cell death in traditional genetic screens. To study WAN-1, Shen *et al*. had to proceed to its partial depletion through the small interfering RNA (siRNA) technology. The partial, non-lethal depletion of WAN-1 strongly inhibited both developmental and germline cell death in *C. elegans*, leading to the persistence of ectopic cells throughout development and to a reduction in the rate of homeostatic cell death, respectively. Accordingly, the ectopic overexpression of WAN-1 could induce apoptosis through a pathway that required CED-3 and CED-4.[3]

At the biochemical level, Shen *et al*.[3] showed that WAN-1 forms a complex with CED-4 and CED-9, and that EGL-1 can disrupt the interaction between CED-9 and WAN-1 (Figure 1). These findings establish the contribution of WAN-1 to the 'core' machinery, and they are reminiscent of the initial molecular studies implicating ANT in mammalian cell death. Indeed, in yeast-two-hybrid screens, Bcl-2 was found to interact with a fragment of the human ANT1 isoform, and Bcl-2 as well other proteins from the Bcl-2 family (such as Bcl-X_L and Bax) were then shown to co-immunoprecipitate with ANT.[35] Subsequent experiments based on highly purified ANT reconstituted into proteoliposomes or planar lipid bilayers revealed that Bcl-2 and Bax act as an allosteric activator and an inhibitor of the antiporter function of ANT (and hence stimulates and reduces ATP/ADP exchange), respectively.[35,36] Moreover, while Bcl-2 was shown to inhibit atractyloside-induced pore formation by ANT, Bax facilitated the permeabilization of artificial membranes containing ANT.[35] Intriguingly, Bax-mediated inhibition of the antiporter activity of ANT (resulting in transient mitochondrial hyperpolarization and alkalinization of the mitochondrial matrix) occurs before (and hence could be discriminated by) cooperative channel formation by Bax and ANT (which—conversely—leads to persistent $\Delta\psi_m$ dissipation and cell death).[36]

It should be noted that genetic evidence suggesting a phylogenetically old contribution of ANT to the cell death machinery has been accumulating in another ancient organism, yeast (*Saccharomyces cerevisiae*), which recently has emerged as a valuable model for investigating the regulation of mitochondrial apoptosis. The deletion of all three ANT-encoding genes (i.e. *aac1*, *aac2* and *aac3*) renders yeast cells resistant against death induced by the overexpression of human Bax[35] and of the HIV-1-encoded protein Vpr.[37] Similarly, Δ*aac1/2/3* yeast cells are protected against cytochrome *c* release and death triggered by acetic acid as well as by the divalent thiol-reactive agent diamide.[38] Moreover, the overexpression of NUC1P (the yeast ortholog of endonuclease G, a mitochondrial intermembrane space protein that, on MOMP, translocates to the nucleus, where it promotes chromatin degradation independently of caspases) reportedly sensitizes *S. cerevisiae* cells to H_2O_2-mediated killing, an effect that is significantly reduced in Δ*aac2* cells and completely abolished in *aac1/2/3* cells.[39]

ANT and Its Homologs in Mammalian Cell Death

There are several closely related ANT isoforms that are encoded by distinct genes, in all species that have been investigated in this respect (human, mouse, *C. elegans* and *S. cerevisiae*). In addition, ANT belongs to the mitochondrial carrier protein (MCP) family (also known as 'solute carrier' family), whose members are involved in the transport of ions and metabolites, mostly across the mitochondrial inner membrane.[40] Despite their close homology, distinct ANT isoforms may have opposed (and perhaps context dependent) roles in cell death. Thus, the overexpression of human ANT1 (which is mainly expressed in the heart and skeletal muscles) and ANT3, but not of ANT2 (which is ubiquitous), can induce apoptosis,[41,42] and the siRNA-mediated knockdown of ANT2 reportedly sensitizes cancer cells to MMP-inducing agents,[43] suggesting that ANT1 and ANT3 are pro-apoptotic proteins whereas ANT2 exerts pro-survival functions. In line with these notions, an interactor of ANT2 (i.e. ANT2-binding protein, ANT2BP) has been recently identified as a protein that is overexpressed in pancreatic ductal adenocarcinoma and is required for the survival of tumor cells.[44] siRNA-mediated knockdown of several ANT isoforms including ANT1 and ANT3 conferred cytoprotection to cisplatin-treated non-small cell lung cancer A549 cells.[45] Human ANT3 has no orthologs in mice, and has been shown to be required for the TNF-induced death of MCF7 breast cancer cells.[46] Murine ANT4, which is only expressed in testicular cells, is essential for germ cell viability *in vivo*,[47] yet has not been investigated with respect to its apoptosis-modulatory function. Intriguingly, ANT4 (which is conserved in mammals yet absent in non-mammalian species) has been indicated as a mitosis-specific protein that is repressed in somatic cells by the E2F6 transcription factor.[48] The mitochondrial phosphate carrier (SLC25A3), another member of the MCP family, has been found to interact with the viral Bcl-2 analog vMIA (encoded by the genome of *Cytomegalovirus*)[49] and to induce apoptosis on overexpression.[50] Accordingly, knockdown of this protein, which can interact with ANT1 and with the voltage-dependent anion channel (VDAC), limited staurosporine (STS)-induced cytochrome *c* release and apoptosis.[50] In the same context (a high-throughput screening for mammalian cell death effectors),[50] also ANT3 and the mitochondrial carrier homologs (MTCHs) 1 and 2 were identified. Thus, ANT1, ANT3, as well as several distinct proteins from the MCP family may exert pro-apoptotic functions.

Proteoliposomes including partially purified permeability transition pore complexes (PTPCs) from mouse liver (which contains both ANT1 and ANT2) or highly purified ANT from rat heart (in which ANT1 is predominant) can be permeabilized *in vitro*, for instance by adding atractyloside, and this

phenomenon can be inhibited by bongkrekate as well as by recombinant Bcl-2 or Bcl-X_L.[35,51] A number of additional apoptotic inducers including Ca^{2+}, ROS, nitric oxide, diamide as well as several experimental anticancer agents permeabilize ANT1-containing (but not ANT-free) liposomes,[52–54] suggesting that ANT is (one of) the protein(s) that might mediate MMP. Intriguingly, it has been suggested that (at least in some experimental settings) ANT1 overexpression might trigger cell death through a PT-independent mechanism relying on the ROS-dependent upregulation/activation of Bax.[55]

Ant1$^{-/-}$ mice develop a mitochondrial (cardio)myopathy, that is associated with increased serum lactate in resting conditions (indicative of metabolic acidosis) and severe exercise intolerance.[56] *Ant1*$^{-/-}$ (cardio)myocytes display dramatic mitochondrial hyperproliferation, which presumably results from a compensatory mechanism triggered by the severe respiratory defect of ANT1-deficient mitochondria.[56] Recently, *ant1*$^{-/-}$ neurons have been ascribed with a near-to-complete resistance to glutamate-, kainate- and etoposide-induced cytotoxicity.[57] Although the phenotype of whole-body *ant2*$^{-/-}$ animals remains to be characterized, *ant4*$^{-/-}$ mice exhibit a significant reduction in testicular size (yet do not manifest any other obvious abnormality).[47] In the mouse liver, tissue-specific double knockout of *ant1* and *ant2* failed to yield a major cell death phenotype,[58] presumably due to a 'rescuing' effect ensured by additional ANT isoforms (or perhaps by other members of the MCP family). Still, hepatocytes deficient for both ANT1 and ANT2 were more resistant to Ca^{2+}-induced PT than their ANT-proficient counterparts,[58] as did mitochondria isolated from ANT1-deficient neurons.[57] As ANT deficiency only elevated the amount of Ca^{2+} required to trigger PT, yet did not confer an absolute protection,[58] it has been a matter of (a perhaps semantic) debate whether ANT is a *bona fide* component or just a 'modulator' of the molecular machinery that permeabilizes mitochondria. It is conceivable that multiple proteins from the MCP family (e.g. ANT1, ANT3, the mitochondrial phosphate carrier, MCHs) contribute to MMP as pore-forming proteins, each acting in response to different thresholds of distinct lethal stimuli (such as Ca^{2+}, ROS, ceramide, etc.). Such a functional redundancy might explain why (at least in some experimental scenarios) pharmacological or genetic inhibition of specific ANT isoforms poorly affects PT-dependent mitochondrial cell death.

In this issue of *Cell Death and Differentiation*, the group of Mauro Piacentini demonstrates that type 2 transglutaminase (TG2) regulates the correct assembly and function of ANT1 *in vitro* and *in vivo*.[59] Malorni *et al*. provide evidence that ANT1 and TG2 physically interact at mitochondria, and that the protein disulfide isomerase activity of TG2 is important to limit ANT1 oligomerization in physiological conditions, thereby controlling the vital function of ANT1 as an ATP/ADP antiporter. Moreover, TG2 appears to be required for (or at least to contribute to) the induction of cell death by multiple triggers, including the glycolysis inhibitor deoxy-D-glucose (DDG) and STS. Intriguingly, *tg2*$^{-/-}$ mouse embryonic fibroblasts were more resistant to DDG- and STS-induced apoptosis than their wild-type counterparts, whereas they failed to display the translocation of Bax to mitochondria and the subsequent interaction of Bax with ANT1. These data suggest that, at least in some experimental settings, TG2 might be mandatory to enable and/or stabilize the association of Bax with ANT1.[59]

An Intricate Interplay Between Distinct Systems of Mitochondrial Permeabilization

According to a widespread point of view, MMP can be the final outcome of two fundamentally distinct phenomena. On one hand, MMP would follow the Bax/Bak-dependent permeabilization of the mitochondrial outer membrane (MOMP), which would be ignited by pro-apoptotic BH3-only proteins (e.g. Bid, Bad) and blocked by anti-apoptotic members of the Bcl-2 family (e.g. Bcl-2, Bcl-X_L). On the other hand, the so-called PT would mainly affect the mitochondrial inner membrane and would be regulated by the PTPC, a supramolecular complex assembled at the contact sites between the mitochondrial outer and inner membranes by the dynamic interaction of multiple proteins including ANT, VDAC and cyclophilin D.[60] (Figure 3). Schematically, MOMP has been preferentially linked to apoptosis and PT to necrosis.[61–63] However, a careful analysis of the data published during the last decade reveals several examples in which Bcl-2-like proteins inhibit PT and necrosis,[64] as well as many instances in which PTPC components (e.g. cyclophilin D) affect MOMP and apoptosis.[65,66] The current literature points indeed to a relevant crosstalk between Bcl-2 family members and constituents of the PTPC.[21] As discussed above, both the ATP/ADP antiporter and the pore-forming activity of ANT can be controlled by pro- and anti-apoptotic proteins of the Bcl-2 family,[36,67] and ANT might activate Bax in an ROS-dependent manner.[55] Moreover, the physical interaction between Bcl-X_L and VDAC1 has been confirmed at the level of NMR solution structure;[68] the pro-apoptotic proteins Bid[69,70] and Bad[71] can induce (at least in some instances) Bax/Bak-independent PT; and cyclophilin D has recently been shown to interact with Bcl-2, thereby augmenting its MOMP-inhibitory capacity.[65] Taken together, these results, as well as the intriguing finding that ANT is part of the 'core' machinery that executes cell death in *C. elegans*, should lead to the reconsideration of the overdidactic distinction between apoptotic MOMP *versus* necrotic PT.

The distinct isoforms of ANT do not only contribute to the assembly/function of the PTPC. At least in yeast, they also interact with respiratory chain 'supercomplexes', together with other MCP family members,[72] as well as with the TIM23 machinery.[73] This suggests that proteins modulating the respiratory chain or the mitochondrial import system might indirectly influence ANT activity and perhaps provide ANT with novel functions. For instance, it has been proposed that the multiprotein interaction among ANT, the mitochondrial phosphate carrier, succinate dehydrogenase and ATP synthase would generate the mitochondrial ATP-sensitive K^+ (mitoK_{ATP}) channel activity that has a central function in the protection of cardiac and neuronal cells against ischemia and apoptosis.[74] In mammalian cells subjected to pro-apoptotic stimuli, the composition of the ANT interactome changes, and in particular the interaction between ANT and Bcl-2 is reduced whereas that between ANT and Bax is enhanced.[60] Similarly, the ANT interactome changes in dying *C. elegans*

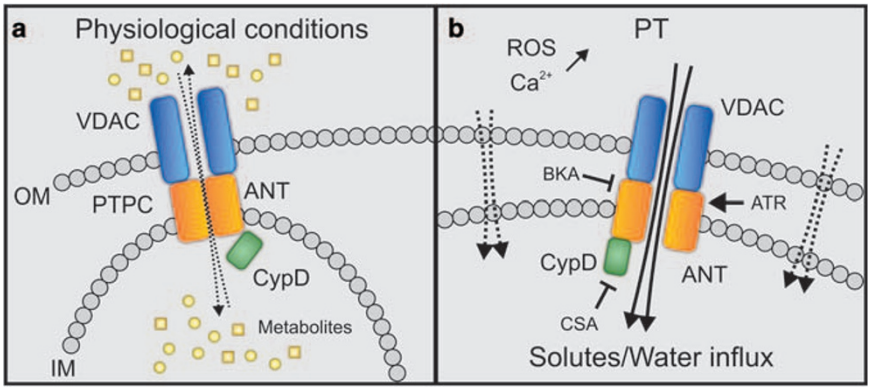

Figure 3 Mitochondrial permeability transition. The permeability transition pore complex (PTPC) is a supramolecular entity assembled at the junctions between the mitochondrial outer and inner membrane (OM and IM, respectively), thanks to the interaction of several proteins including the adenine nucleotide translocase (ANT, in the IM), the voltage-dependent anion channel (VDAC, in the OM) and cyclophilin D (CypD, in the mitochondrial matrix). (**a**) In healthy cells, the PTPC displays a 'flickering' conformation, which allows for the physiological exchange of small metabolites between the cytosol and the mitochondrial matrix. (**b**) In response to multiple signals including reactive oxygen species (ROS) overgeneration and Ca^{2+} overload, the PTPC assumes a high-conductance state, allowing for the unregulated entry of solutes (and hence water, driven by osmotic forces) into the mitochondrial matrix. This phenomenon is known as permeability transition (PT) and leads to the swelling of mitochondria eventually followed by OM breakdown. Pharmacological agents that bind to specific PTPC subunits are known to induce (e.g. atractyloside, ATR) or inhibit (e.g. bongkrekate, BKA, cyclosporine A, CSA) PT. Please see the text for further details

cells.[3] Thus, one might envision a molecular pool game in which distinct pro- and anti-apoptotic signals converge on mitochondria and affect the composition of several supramolecular complexes, as well as the conformation of numerous pore-forming proteins, thereby favoring or inhibiting lethal MMP. The rules governing this game require urgent elucidation.

Concluding Remarks

The regulation of cell death in mammals, and in particular the mitochondrial battleground on which multiple vital and lethal forces combat, undoubtedly is more complex than that of model organisms like *C. elegans* and *S. cerevisiae.* Nevertheless, most cell death investigators formulate questions in which simple, all-exclusive mechanisms are sought. For instance, dozens of experiments have been designed and performed to elucidate which mitochondrial lipids and proteins are required for Bax-mediated MOMP or Bcl-2-dependent cytoprotection. Most likely, the molecular protagonists of the fight between death and life use multiple (rather than simple) strategies to induce or inhibit MOMP. Thus, it is conceivable that Bax itself and/or Bax-like proteins induce MOMP by forming supramolecular homo-oligomeric complexes in the mitochondrial outer membrane, yet also interact with sessile mitochondrial proteins (such as ANT, VDAC and perhaps others[75]) to facilitate PT and/or to form mixed protein complexes with membrane-permeabilizing properties. Similarly, some BH3-only proteins may induce the activation of pro-apoptotic multidomain proteins of the Bcl-2 family and also promote Bax/Bak-independent PT. Along the same line, anti-apoptotic Bcl-2 homologs likewise seal mitochondrial membranes not only by sequestering/inhibiting their pro-apoptotic counterparts but also by interacting with other proteins including ANT and VDAC. Our feeling is that this view might accommodate much of the controversial findings in the field of mitochondrial cell death research, at the same time that it might provide the theoretical scaffold for further mechanism-based and translational research on this important subject.

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