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Minireview

Mitochondrial effectors in caspase-independent cell death

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Abstract Activation of caspases is recognized as a key element in the apoptotic process. However, new evidence is drawing attention to the emergent role of cell death pathways where caspases are not involved. Recent advances in the molecular understanding of these new ways to die, called caspase-independent, have revealed that mitochondria play an important role via the release of proapoptotic proteins. The purpose of this review is to integrate, from a biological and structural point of view, the most recent advances in the knowledge of the main mitochondrial proapoptotic proteins involved in this cell death cascade. The origin of programmed cell death is discussed through these strongly conserved effectors.

Key words: Apoptosis-inducing factor; Apoptosis; Caspase-independent cell death; Endonuclease G; Omi/HtrA2; Mitochondrion

1. Introduction

In multicellular organisms, programmed cell death (PCD, apoptosis) is a genetically regulated mechanism that plays a crucial role in the control of normal development and in the regulation of tissue homeostasis [1]. Failure to accurately undergo apoptosis can cause severe anomalies, ranging from autoimmune diseases to cancer [2]. In the last decade, much of the attention in the study of PCD was focused on caspases, a family of cysteine proteases specifically activated in apoptotic cells [3]. However, cell death can still occur even when the caspase cascade is blocked [4]. This fact has revealed the existence of alternative pathway(s) defined as caspase-independent [5,6].

The induction of mitochondrial membrane permeabilization (MMP), which is controlled by the Bcl-2 family of proteins, is a critical event in apoptosis. Most pathways upstream of MMP are caspase-independent, and both caspase-dependent and caspase-independent paths become possible after mitochondrial damage. In classical apoptosis (caspase-dependent), after MMP induction cytochrome *c* redistributes from mitochondria to cytosol to activate caspase-9, in collaboration with ATP and the cytosolic factor Apaf-1 [3]. In other mechanisms implicating caspases and mitochondria, caspase-inhibitory factors are separated from their physiological partners by mitochondrial proteins such as Smac/DIABLO[1] or Omi/HtrA2, resulting in caspase activation [7]. The intermembrane space of mitochondria also contains other proteins, such as AIF and EndoG, which can provoke PCD in a caspase-independent manner [8]. Finally, Omi/HtrA2 can build a shuttle between caspase-dependent and -independent PCD through its serine protease activity [9]. Therefore, the mitochondrion is not only the cell's powerhouse, but also a weapon store where a cocktail of caspase-dependent or -independent proapoptotic proteins are sequestered until death is triggered [10,11]. The present review aims to integrate the current knowledge about the mitochondrial molecules involved in caspase-independent cell death (CICD), a highly orchestrated form of cell death.

2. Apoptosis-inducing factor

AIF was identified and cloned in 1999 as a protein released from the mitochondrial intermembrane space during the apoptotic process [12]. Initial studies have shown that upon an apoptotic stimulus AIF translocates, in a Bcl-2-controlled fashion, from mitochondria to cytosol and further to the nucleus where it triggers chromatin condensation as well as large-scale (~50 kb) DNA fragmentation [12]. AIF has also a direct effect on both isolated nuclei and purified mitochondria where it provokes the release of caspase-9, thereby suggesting a possible link between AIF and the caspase-dependent pathway [12]. The physiological relevance of AIF was emphasized in the work published in 2001 by Joza et al. [13]. In this paper the authors showed that *aif*$^{-/Y}$ embryonic stem cells were unable to form cystic embryonic bodies. This fact suggests an essential function of AIF in mouse development [13].

AIF is expressed as a precursor of 67 kDa. This form is addressed and compartmentalized in mitochondria by two mitochondrial localization sequences located within the N-ter-

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Abbreviations: AIF, apoptosis-inducing factor; AMID, AIF-homologous mitochondrion-associated inducer of death; EndoG, endonuclease G; HSpin1, human homolog of the *Drosophila* spin gene product; HtrA2, high temperature requirement protein A2; PRG3, p53-responsive gene 3; Smac/DIABLO, second mitochondria-derived activator of caspase/direct IAP binding protein with low p*I*; WOX, WW domain-containing oxidoreductase

[1] For explanation of protein names see the list of abbreviations.

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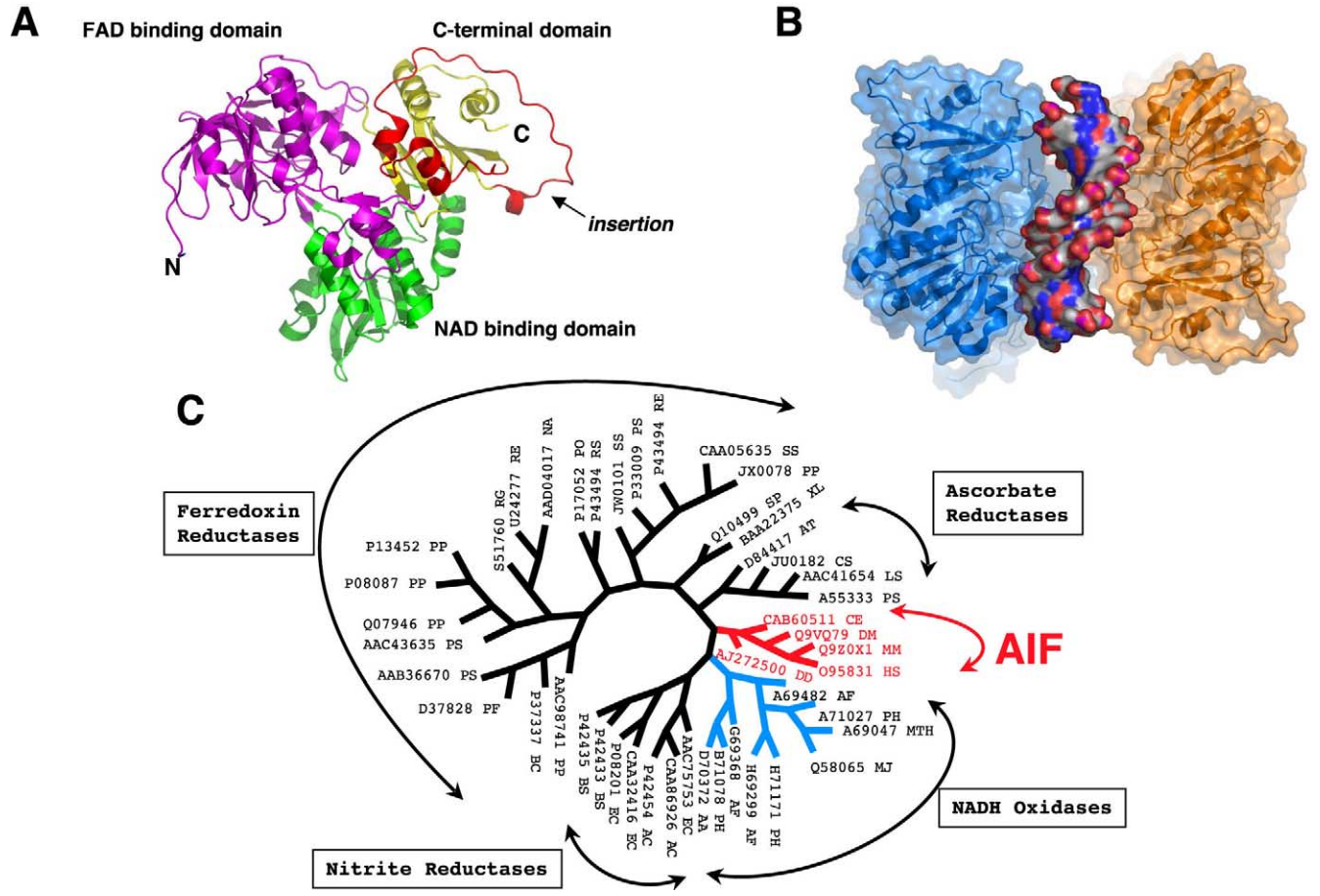

Fig. 1. A: Ribbon structure of AIF showing the three domains of the protein: FAD-binding domain in magenta, NAD-binding domain in green, and C-terminal domain in yellow. B: Model of the theoretical interaction between the dimeric form of AIF and the DNA (id 1GV4). C: Phylogenetic tree representing the relationship between AIF and other oxidoreductases from different species. Note the proximity of the AIF family (red branch) to the NADH-oxidase family from Archaea (blue branch). The PIR accession codes are enumerated following the abbreviation of each specie: AA: *Aquifex aeolicus*; AC: *Acinetobacter calcoaceticus*; AF: *Archaeoglobus fulgidus*; AT: *Arabidopsis thaliana*; BC: *Burkholderia cepacia*; BS: *Bacillus subtilis*; CE: *Caenorhabditis elegans*; DD: *Dictyostelium discoideum*; DM: *Drosophila melanogaster*; EC: *Escherichia coli*; HS: *Homo sapiens*; LS: *Lycopersicon esculentum*; MJ: *Methanocaldococcus jannaschii*; MM: *Mus musculus*; MTH: *Methanobacterium thermoautotrophicum*; NA: *Novosphingobium aromaticivorans*; PF: *Pseudomonas fluorescens*; PH: *Pyrococcus horikoshii*; PO: *Pseudomonas oleovorans*; PP: *Pseudomonas putida*; PS: *Pseudomonas* sp.; PSA: *Pisum sativum*; SP: *Schizosaccharomyces pombe*; SS: *Sphingomonas* sp.; RE: *Rhodococcus erythropolis*; RG: *Rhodococcus globerulus*; XL: *Xenopus laevis*.

minal prodomain. Once in mitochondria, the full-length AIF is processed and the prodomain removed, giving rise to a mature form of ~57 kDa [12]. AIF consists of three structural domains: (i) FAD-binding domain, (ii) NAD-binding domain, and (iii) C-terminal domain (Fig. 1A) [14]. The oxidoreductase part of AIF (composed of both NAD- and FAD-binding moieties) confers an electron transfer activity to the protein that has been fully confirmed in functional assays. In vitro, AIF produces superoxide free radicals as it exhibits NADH oxidase activity catalyzing the reduction of O_2 to $O_2^{\bullet-}$ [15]. The measured rate of AIF electron transfer, from NADH to O_2, is very low [15]. If this rate is similar inside the mitochondria, it is difficult to conceive both the natural electron acceptor of AIF and the physiological relevance of the redox function of the protein. However, the compartmentalization of the protein in the mitochondrion could completely change this redox behavior. The ability of AIF to produce superoxide free radicals apparently contrasts with the data obtained by Ackerman's group in the Harlequin mouse [16]. In this model, cerebellar granule cells obtained from Harlequin mutant mice were more susceptible to peroxide-induced apoptosis when compared with cerebellar granule cells from wild-type mice. Retroviral transfection of AIF eliminates this difference, leading the authors to suggest that AIF acts as a free radical scavenger to prevent apoptosis [16]. Then, what is the true redox function of AIF, promoter or scavenger of free radicals? Probably both, as it seems logical to expect AIF behavior to vary according to the protein location. On the one hand, AIF could accomplish some free radical scavenger activity inside mitochondria either via the reduction of a yet unidentified compound present in the intermembrane space or by taking part in the mitochondrial electron transfer chain. This last idea is supported by a recent proteomic study that identified the presence of AIF in a purified fraction of the complex IV of the mitochondrial oxidative phosphorylation system [17]. However, many traditional experiments carried out with submitochondrial particles, proteoliposomes or purified enzyme did not consider AIF to have an important role in the mitochondrial cytochrome oxidase activity. On the other hand, a different behavior of AIF seems possible when the

protein is released from mitochondria. Once in the cytosol, it is feasible to believe that AIF could associate with different partners to produce the free radicals observed in the in vitro assays. In any case, in vivo models such as the Harlequin mouse and the *aif*$^{-/Y}$ embryonic stem cells are ideal tools to investigate the importance of AIF in cell survival. Indeed, it is possible to compare the respiratory pathways and the electron chain transport occurring in mitochondria purified from Harlequin and wild-type mice. The same study, also possible in *aif*$^{-/Y}$ and *aif*$^{+/Y}$ embryonic stem cells, can definitively close this still theoretical issue.

The C-terminal domain of AIF is the most intriguing part of the protein, given the absence of significant homology with any other protein. Deletion of this domain abrogates the apoptogenic activity of AIF, suggesting an essential role in the death function [12]. A structural analysis of the C-terminal domain of AIF showed that a long insertion resulting in a flexible loop resides in this part of the molecule. This region contains a PEST motif (usually involved in processes of protein degradation) that could explain the disappearance of AIF from the cytosol observed in activated T lymphocytes [18]. Interestingly, in the AIF form of *Dictyostelium discoideum*, the PEST motif switches over the N-terminal part of the molecule [19]. In addition, the extended structure of the C-terminal loop could also suggest a potential binding region for some chaperones, such as Hsp70, which has been shown to interact with AIF and impair its apoptotic function [20]. However, a recent work suggests that Hsp70 interacts with the N-terminal domain of the protein [21]. In this region, two amino acids that are present in the human protein, R192 and K194, seem to be essential for the link [21]. Intriguingly, the C-terminal region of AIF also contains an RNA-binding motif (aa 549–567) which is typical of some ribosomal proteins and suggests a possible binding of AIF to RNA. Unlike this theoretical AIF–RNA interaction, the AIF–DNA interaction has been recently demonstrated [22]. In a first model, structural analysis of monomeric human AIF reveals a strong positive electrostatic charge that might be involved in the interface of interaction with DNA [22]. The interface begins from a pocket between NAD and the C-terminus, and extends to one face of the C-terminal domain. In a parallel work, mouse AIF was crystallized in a dimeric form that creates a deep groove where some of the best conserved basic residues are located [14]. Given its dimensions, we are tempted to speculate that this groove could serve to accommodate a double-stranded DNA molecule (Fig. 1B). Under these conditions, the recruitment of other potential partners such as nucleases, topoisomerases, or cyclophilins might help AIF to complete its DNA degradation function.

Summing up, AIF is a dual-function protein that deals with events of life and death. Confined in mitochondria from healthy cells, AIF could be essential for cell survival through its redox function, as suggested by the work performed in the Harlequin mouse. In contrast, when AIF is released from mitochondria in apoptotic conditions, it triggers cell death either directly, through interaction with DNA, or indirectly, through reactive oxygen species production [23].

3. Endonuclease G

Eukaryotic EndoG is a divalent cation-dependent endonuclease described in chicken erythrocytes in 1987 [24]. EndoG is encoded by a nuclear gene and is mainly associated with functions in DNA repair or mitochondrial DNA (mtDNA) duplication. Their prokaryotic homologs has been implicated in metabolic scavenging functions and virulence.

EndoG is a sequence-unspecific DNase that shows great affinity for kinked DNA at low levels. The degradation of DNA produced by this enzyme gives rise to fragments with nicks at 5′-P and 3′-OH ends. In addition, EndoG also has RNase activity, which generates the RNA primers required by DNA polymerase γ to initiate replication of mtDNA.

Other than its function in mtDNA replication, a new role in the apoptotic process has been assigned to EndoG. Two elegant studies disclosed EndoG's 'double life', as it is the case for other apoptogenic proteins (i.e. AIF, cytochrome *c*, and Omi/HtrA2) [25,26]. These works demonstrated that upon apoptotic stimulus (including tBid, Bax, calcium and chemotherapeutic drugs), EndoG translocates from mitochondria to nucleus, where extensively degrades nuclear DNA into oligonucleosomal fragments, similar to those generated by the caspase effector CAD (caspase-activated DNase). These works also demonstrated that CAD and EndoG activities are independent, since EndoG does not require prior caspase processing for its activation. Intriguingly, recent results suggest that caspases seem to have some role on the translocation of EndoG (and AIF) from mitochondria to cytosol through an unknown mechanism regulated by the proapoptotic members of the Bcl-2 family Bax and Bak [27]. This work provided another link between the Bcl-2 family of proteins, mitochondria and their control of caspase-dependent and -independent cell death pathways.

EndoG, which belongs to an important family of Mg^{2+}-dependent nucleases [28] (Fig. 2A), is synthesized as a precursor of ~30 kDa. After mitochondrial import, the N-terminal region of 48 amino acids is removed yielding the mature form. Structural studies in the homologous of the endonuclease from *Serratia marcescens* revealed a homodimer with two disulfide bonds that provide high stability and resistance to reduction and to chaotropic agents [28] (Fig. 2). The homolog of EndoG is built in an original folding that consists of a central six-stranded antiparallel β-sheet flanked by α-helices on both sides (Fig. 2B). Each monomer contains a catalytic site that is highly conserved throughout all the species studied (Fig. 2A). Enzymological analysis of this homolog reveals two critical residues for its nuclease activity: (i) H89, which could act as general base activating a nucleophilic water molecule responsible for the DNA cleavage, and (ii) N119, which directly binds Mg^{2+} (Fig. 2B). Point mutations in each one of these residues abrogate the nuclease activity [28].

Genetic studies performed in *Caenorhabditis elegans* provide clear evidence for an important role of the EndoG ortholog *cps-6* in the breakdown of DNA during developmental cell death [26]. Using RNAi techniques (or reduction of *cps-6* activity by a genetic approach) the authors showed that during *C. elegans* development, the appearance of cell corpses was delayed by the downexpression of CPS-6. Another important issue involved in a thorough understanding of CICD emerges from a recent work also performed in *C. elegans* [8]. In this organism, CPS-6 binds and acts synergistically with WAH-1 (the AIF ortholog in *C. elegans*). These findings demonstrate that AIF and EndoG can cooperate, thereby highlighting the importance of both molecules in PCD execution.

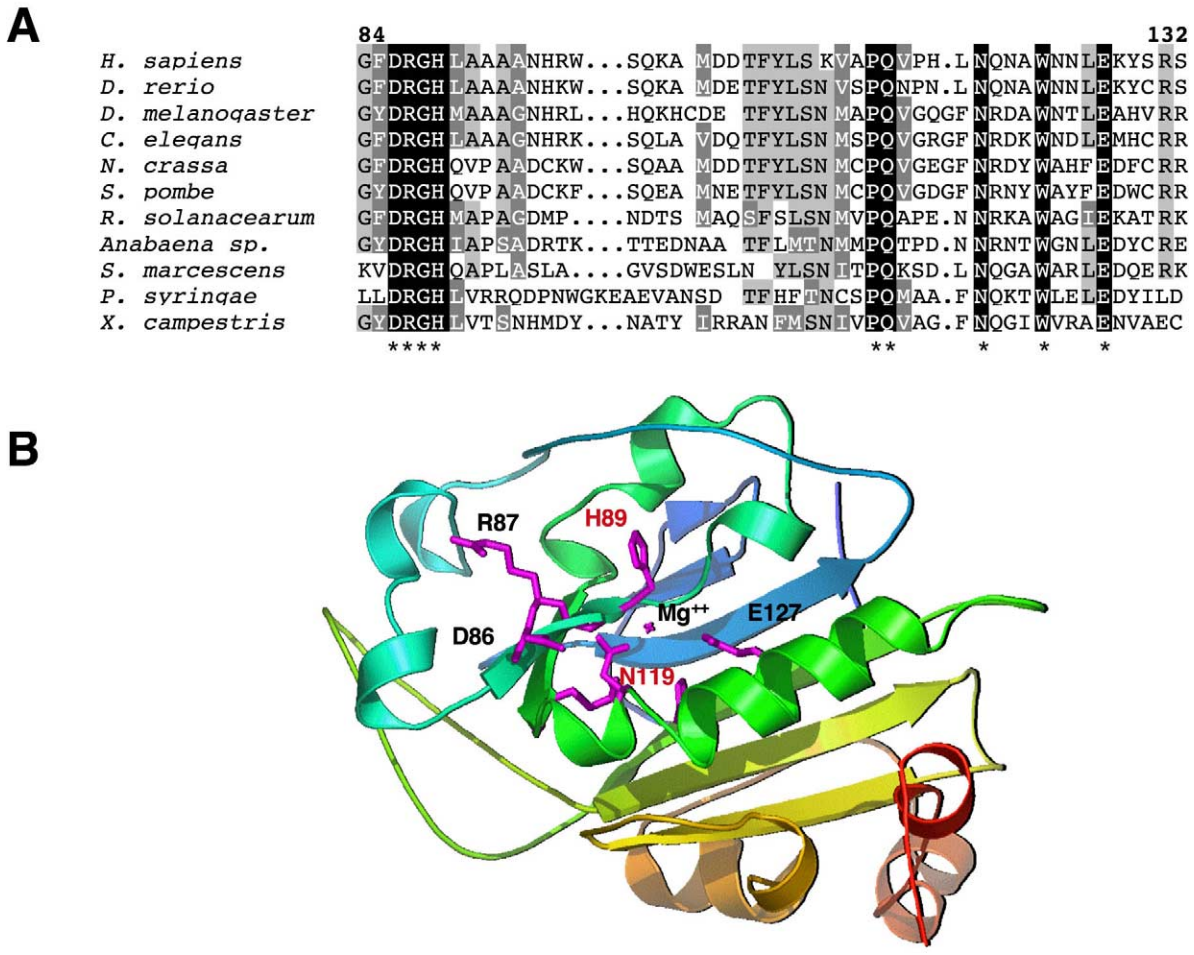

Fig. 2. A: Amino acid sequence alignment of nucleases homologous to EndoG in the region containing the catalytic site. Note the strict conservation of the essential residues. B: Ribbon diagram of the homolog from *Serratia marcescens*. Catalytic residues are colored in magenta.

4. Omi/HtrA2, an effector with dual apoptotic activity

Identified in 2000, Omi/HtrA2 is a 50 kDa mammalian serine protease that shares significant homology with the bacterial endoprotease HtrA [29,30]. In bacteria, the protein has a chaperone function and helps to degrade or refold proteins in the periplasmic space. In mammals, Omi/HtrA2 is a ubiquitous protein with a dual proapoptotic function [31].

Omi/HtrA2 is a nuclear-encoded mitochondrial protein synthesized as a proenzyme of 458 amino acids. After mitochondrial import, 133 N-terminal residues of the protein are removed to generate an active form of 36 kDa. The resulting molecule exposes an amino-terminus (AVPS motif) that shares high homology with *Drosophila* Grim, Hid, Reaper, and mammalian Smac/DIABLO proteins. The AVPS tetrapeptide motif consists in a short stretch of hydrophobic amino acids that competitively bind the baculoviral IAP repeat domain of inhibitor of apoptosis proteins (IAPs) [31]. In physiological conditions, Omi/HtrA2 is confined in mitochondria and kept away from IAPs. Upon apoptotic stimulus, the mature form is released to the cytoplasm to bind IAPs. The interaction between Omi/HtrA2 and IAP disrupts the IAP–caspase inhibitory complex and results in caspase activation. In this sense, the caspase activation mechanism prompted by Omi/HtrA2 resembles to that triggered by Smac/DIABLO, which also binds IAPs and subsequently activates caspase-9 [32,33]. Omi/HtrA2 can also induce apoptosis in a caspase-independent manner that seems linked to its serine protease activity [31].

The topology of mature Omi/HtrA2 comprises seven α-helices and 19 β-strands, forming two domains: the catalytic serine protease domain and the PDZ domain (Fig. 3A). The catalytic domain of Omi/HtrA2 is located in the central region of the molecule, while the PDZ domain, which regulates the serine protease activity, is located in the C-terminal region. PDZ domains are known to bind four or five residues of their target proteins through a hydrophobic cleft composed of a β-strand, an α-helix and a loop that binds the peptide carboxylate group. The crystal structure of Omi/HtrA2 has also revealed that the formation of a pyramid-shaped homotrimer is a prerequisite for the caspase-independent function of the protein [34] (Fig. 3B). Mutational analysis confirms that monomeric Omi/HtrA2 is unable to induce cell death when its serine protease activity is seriously impaired. A model for Omi/HtrA2-induced apoptosis suggests that the PDZ domains of oligomeric Omi/HtrA2 block access to the catalytic site until recognition of the substrate. Contact with the partner provokes a conformational change that induces exposure of the substrate-binding sites of the protease and results in a cleavage of the substrate [34] (Fig. 3B).

The functional significance of Omi/HtrA2 was greatly underlined after a recent work that examined its role in the

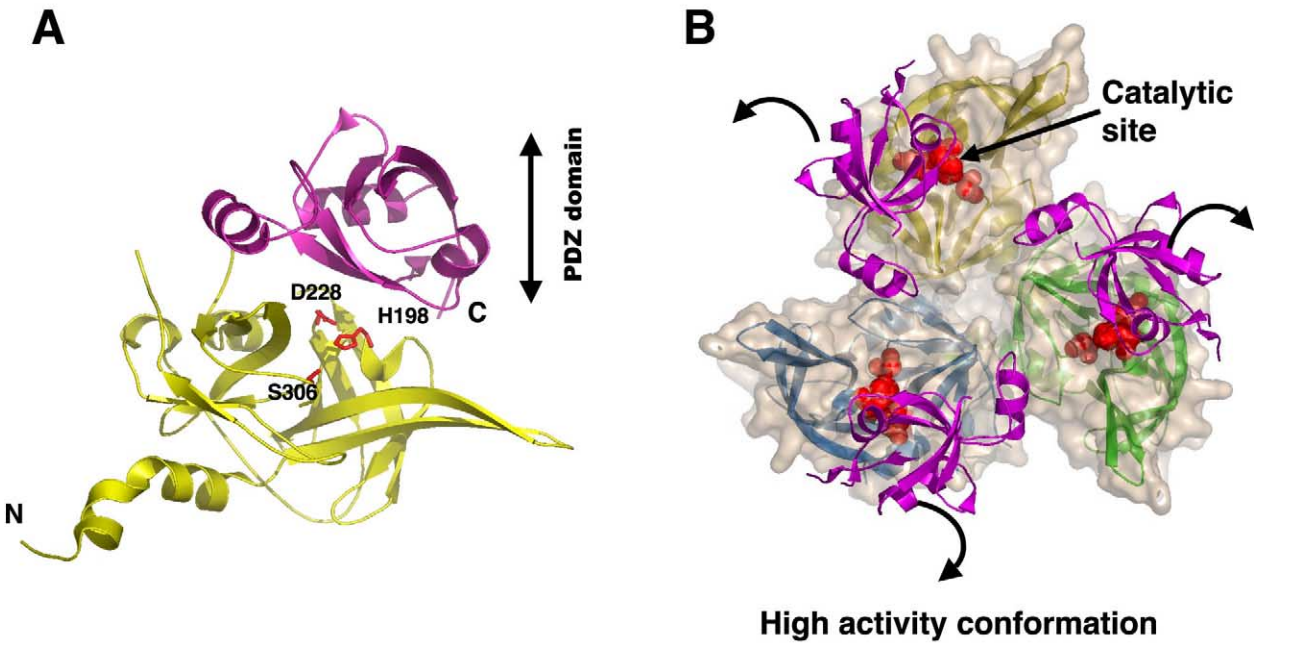

Fig. 3. A: Ribbon structure of Omi/HtrA2. The catalytic residues are represented in red and the PDZ domain in magenta. B: Model for the trimeric form of Omi/HtrA2. The IAP-binding domains of each of the three monomers are colored in yellow, blue and green, respectively. All PDZ domains are colored in magenta. Recognition of the substrate would drive a conformational change where the PDZ domains open up (arrows) to expose the catalytic site (red balls), resulting in cleavage of the substrate.

mouse mutant *mnd2* (motor neuron degeneration 2) [35]. In this work, the authors showed that the point mutation S276C provokes neuronal degeneration as well as juvenile lethality. Loss of the caspase-independent activity of Omi/HtrA2 also provokes mitochondrial alterations and increases sensitivity to cellular stress. In addition, two recent studies could shed considerable light on the mechanism employed by this protein to induce CICD. On the one hand, a specific inhibitor of their caspase-independent activity, named ucf-101, has been identified [36]. This inhibitor can be used as a tool to differentiate the two proapoptotic activities of the protein and their contribution to PCD. On the other hand, the optimal substrate sequence for cleavage by Omi/HtrA2 has been identified: a crucial finding to better understand the regulation and the function of this dual protein [37].

5. New arrivals in the family of mitochondrial caspase-independent effectors

The avalanche of fruitful investigations in the domain of PCD constantly adds new proteins to the list of molecules involved in caspase-independent events. In 2001 Chang et al. reported a new proapoptotic oxidoreductase activity that was named WOX1 [38]. This protein, also called WWOX or FOR [39] [40], bears two WW domains in its N-terminal sequence. Eight spliced forms have been described, of which WOX1 (WWOX, FOR2, or v1) is the full-length protein [41]. This protein exhibits many interesting properties. For example, WOX1 enhances the tumor necrosis factor-α (TNFα) apoptotic responsiveness via downregulation of antiapoptotic Bcl-2 molecules, upregulation of proapoptotic p53 or by interaction with molecules downstream of the TNF receptor, such as TRAF2 and TRADD. A portion of WOX1 is confined in mitochondria from fibroblasts in physiological conditions. Upon apoptotic stimuli (i.e. TNFα, staurosporine), WOX1 is released from mitochondria to the cytosol and to the nucleus, a translocation that seems to be dependent on the phosphorylation at Y33. In addition, WOX1 physically interacts with p53 and induces apoptosis in a synergistic manner, since siRNA knockdown of WOX1 abrogates p53 apoptosis. The proposed interface of binding involves the interaction of the WW domains of WOX1 with the proline-rich region of p53. Besides, phosphorylation of S46 in p53 and both Y33 and Y61 in WOX1 is required for such interaction to take place. In addition, the discovery that WOX1 can also interact with c-Jun N-terminal kinase 1 (JNK1) introduced a high level of complexity to this system. In fact, UV stress induces WOX1 phosphorylation at Y33 as well as activation of p53 and JNK1, suggesting the formation of a heterotrimeric complex of WOX1/JNK1/p53 [41]. The diversity of pathways in which WOX1 is involved turns them into a very attractive field of research. Hopefully, future works will respond to the unanswered questions concerning the involvement of WOX1 in redox functions, for example, or the relationship among the different WOX spliced forms.

In 2002 two homologs of the mature form of AIF were simultaneously reported: AMID [42] and PRG3 [43]. Both proteins share an identical amino acid sequence, but the authors have reported significant differences. AMID is mainly localized in the outer mitochondrial membrane, while PRG3 resides in the cytosol. In addition, AMID-induced apoptosis is independent of caspases and p53, while PRG3 expression triggers p53- and caspase-dependent cell death. A more detailed analysis seems necessary to determine the relationship among the different AIF spliced forms and their physiological relevance.

Very recently, a human homolog of the *Drosophila* spin gene product, called HSpin1, was identified [44]. This protein is localized in mitochondria and released to cytosol after TNFα treatment. HSpin1 binds to Bcl-2 and Bcl-Xl probably

to modulate their antiapoptotic effects. The authors also found that the HSpin1-induced cell death was abrogated by the necrotic inhibitor pyrrolidine dithiocarbamate, but not by the broad-range caspase inhibitor z-VAD.fmk. This fact suggests an atypical caspase-independent apoptosis with some necrosis-like characteristics. Furthermore, HSpin1 expression can induce accumulation of monodansylcadaverine (a specific marker for autophagic vacuoles) as well as maturation of cathepsin D. Taken together, these data strongly suggest that HSpin1 is involved in a necrotic-like or autophagic cell death pathway that acts in a caspase-independent manner.

6. Evolutionary considerations of caspase-independent cell death

Evolution of life on earth underwent critical transitions that gave rise to the origin of eukaryotes and the advent of multicellularity [45]. These evolutionary steps involved the development of innovative functional mechanisms such as cell cycle regulation or PCD. Initial phylogenetic studies of apoptosis basically suggest that caspases are proteins from highly evolved apoptotic systems. In contrast, caspase-independent molecules belong to older systems that emerged before the birth of the eukaryotic cell. The recent discovery of metacaspases and paracaspases, also present in prokaryotes, could contravene this hypothesis if their implication in cell death processes is confirmed.

AIF is a highly conserved protein ubiquitously present in all primary kingdoms, Bacteria, Archaea and Eucaryota (Fig. 1C). Its phylogenetic study has some constraints because AIF orthologs have a well-recognized pyridine nucleotide–disulfide oxidoreductase domain, found in a large number of non-apoptotic proteins, which can mask or alter the scores of the apoptotic motifs in databases. As opposed to EndoG and Omi/HtrA2, AIF is strongly represented in Archaea. Among eukaryotes, a strong homology is seen in plant ascorbate reductases (i.e. *Arabidopsis thaliana*, *Cucumis sativus* or *Lycopersicon esculentum*) [46] (Fig. 1C). Intriguingly, as is the case in mammalian AIFs, dehydroascorbate reductase activity has been reported to redistribute from mitochondria to the cytosol in the dark-induced senescence of *Pisum sativum* [46]. AIF also has a highly significant homology with different families of oxidoreductases, from Archaea and Bacteria to invertebrates and vertebrates. In a recent work, Koonin and Aravind [47] concluded that AIF is the unique known element of the apoptotic machinery that obeys the rules of standard evolution. Academically, this means that the *aif* gene is inherited from the last universal common ancestor and follows the tree topology with the primary radiation of the archaeo-eukaryotic and bacterial clades. Basically, if the implication of AIF orthologs in death processes is confirmed, the apoptosis triggered by AIF could represent the oldest way to die.

From a phylogenetic point of view, EndoG is a strongly conserved protein present in eukaryotes and prokaryotes, such as Proteobacteria and Cyanobacteria, but absent in Archaea. Although the implication of EndoG in the apoptotic process has been fully demonstrated only in mammals and *C. elegans*, ladders of fragmented DNA have been observed in their respective EndoG orthologs in plants, fungi and protozoa. Further studies would be necessary to determine if the nuclease activity of these orthologs is involved in cell death.

Phylogenetic analysis of the HtrA2 family strongly supports the mitochondrial endosymbiosis theory and a mitochondrial origin of the eukaryotic HtrA2-like proteases. As a protein with ancient evolutionary origins, it probably has an important housekeeping function in protein refolding and in degradation within mitochondria. Omi/HtrA2 exhibits an evolutionary pattern that is similar to EndoG's. According to the studies of Koonin and Aravind, Omi/HtrA2 is present in Bacteria with the exception of mycoplasms [47]. Omi/HtrA2, like EndoG, is also absent in Archaea but present in α-Proteobacteria, following the most widely accepted model of the mitochondrial origin of eukaryotic proteins [48].

Despite their well-differentiated mechanisms, caspase-dependent and -independent pathways may operate together (or in parallel) in eukaryotic cells. This apparent redundancy ensures the cell suicide once the decision of death has been made. More studies in different species are required in order to understand the evolution of these pathways. Particularly, the study of their putative implication in bacterial pathogenesis is a very promising field of research. It would be very interesting to study the functional implication of the homologs of caspase-independent or caspase-dependent systems (paracaspases, metacaspases) in the mechanisms of cell suicide or bacteria-induced toxicity. The evolutionary cell death scenario, set up through the structural analysis of the mitochondrial proapoptotic proteins, suggests that the eukaryotic cell originated when bacteria were captured by a proto-eukaryotic cell. The modern eukaryotic cell seems to be the legacy of this ancestral symbiotic relationship between the proteobacterium and its host: today both are destined to live together in mutual cooperation for life and death.

7. Concluding remarks

The existence of multiple programs of cell death is strongly supported by the vast amount of information disclosed in recent years. It is clear that CICD effectors may also perform critical functions for cell survival. In this context, the general analysis of caspase-independent pathways of death is a new challenge for researchers. Further studies are necessary to understand their biological relevance and their implication in pathological processes. In any case, it seems clear that a full understanding of the regulation of CICD could provide new means of improving current diagnosis and promoting conceptual advances for the design of new therapeutic strategies.

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