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News and Commentary

Jekyll and Hyde; changing places, changing faces

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Reviews in *Cell Death and Differentiation* are not often concerned with proteases other than caspases, particularly not bacterial ones, however the latest findings on a novel pro-apoptotic molecule, a human homologue of *E. coli*, HtrA, suggest that this might soon change. Bacterial DegP/HtrA is a heat shock inducible periplasmic serine protease that is necessary for bacterial tolerance to thermal, osmotic and oxidative stress. The mammalian homologue HtrA2/Omi has recently been shown to interact with IAPs in an analogous manner to other IAP antagonists, such as Diablo/Smac.[1–4] In a novel twist however, not only does HtrA2 promote cell death by antagonizing IAPs but it can also accelerate cell death in a caspase independent fashion through its function as a serine protease.

Like Diablo/Smac,[5,6] HtrA2 is: (a) directed to the mitochondria by an N-terminal mitochondrial import sequence; (b) must be N-terminally processed to expose the conserved IAP binding motif;[7,8] (c) is located predominantly in the intermembrane space of the mitochondria; and (d) is released from the mitochondria, into the cytosol following apoptotic stress where it can interact with IAPs via their baculoviral IAP repeats (BIRs).[1–4] HtrA2 interacts with mammalian XIAP, cIAP-1, cIAP-2 and baculoviral OpIAP, but not survivin, and can interact with both BIR2 and BIR3 of XIAP but not BIR1.[1–4] HtrA2 binds with higher affinity to BIR2 than BIR3, whereas the reverse is true for Diablo, suggesting that it may function more efficiently to antagonize XIAP inhibition of caspase 3, than of caspase 9.[2] Nevertheless, the same critical residues within the grooves of BIR2 and 3 shown to be important for Diablo interaction[9,10] are also important for interaction with HtrA2.[1–4] Furthermore, like Diablo and cytochrome *c*, release of HtrA2 from the mitochondria can be inhibited by Bcl-2 overexpression.[2,11]

HtrA2 can antagonize IAP inhibition of caspase activity *in vitro* and can promote apoptosis in cells exposed to UV, staurosporine and the TNF family member TRAIL.[1–4] Its role in promoting cell death has been demonstrated by: (a) enhanced cell death responses to apoptotic stimuli in transfected cells transiently expressing HtrA2 and (b) reduced cell death responses of cells in which endogenous expression was eliminated by RNA interference.[4] In contrast to Diablo/Smac, however, HtrA2 has a dual mechanism for promoting cell death that utilizes not only its ability to antagonize IAPs but also its function as a serine protease.

When a modified form of HtrA2 without the leader sequence is expressed in the cytoplasm, it induces apoptosis that is independent of both IAP binding activity and caspase activity, but requires the serine protease activity of HtrA2.[1–4] However, unlike the cytoplasmically expressed form, catalytically active mitochondrial HtrA2 does not induce cell death unless it is released from the mitochondria in response to apoptotic insults such as UV irradiation.

Separating the two pro-death functions of HtrA2 while maintaining correct localization is complicated by an apparent requirement for HtrA2 protease activity in autoprocessing.[12,13] One approach used to overcome this problem has been to employ Diablo/Smac-HtrA2 chimeric proteins comprising the N-terminal mitochondrial import sequence of Diablo/Smac fused to the N-terminus of processed HtrA2 or HtrA2 mutants, thereby ensuring appropriate localization and processing.[2] This enabled expression of mutants that were either catalytically inactive but contained the correct IAP interaction motif, catalytically active but with point mutations in the IAP interaction motif, or both catalytically inactive and non-IAP interacting. These mutants established that HtrA2 promotes cell death via both of its 'activities', i.e. double-mutants that were catalytically inactive and unable to antagonize IAPs did not promote cell death, whereas single-mutants did. A similar approach was used to demonstrate the role of HtrA2 as an IAP antagonist in responses to TRAIL. In this case, a fusion comprising GFP and processed, catalytically inactive HtrA2, separated by a caspase 8 cleavage site, became correctly processed following caspase 8 activation revealing its IAP binding motif, and enhancing TRAIL induced cell death.[3]

Interestingly, the bacterial protein, HtrA, in addition to its protease function, can also function as a chaperone, with the switch from chaperone to protease occurring at elevated temperatures.[14] The chaperone activity of *E. coli* HtrA is localized to the protease domain but does not require the catalytic serine residue.[14] Mammalian HtrA2 also shows a slight increase in proteolytic activity from 37°C to 45°C and in response to kidney ischemia/reperfusion,[12,15] but its potential as a chaperone has not yet been investigated.

HtrA is essential for *E. coli* to survive thermal, oxidative and pH stress. By mutating the catalytic serine residue of HtrA it was possible to separate the chaperone function from the protease function and show that thermotolerance can be rescued by the chaperone function of HtrA alone,[14] although the contrary was observed by Skorko-Glonek *et al*.[16] From a bacterial perspective, at higher temperatures it might make more sense to cut its losses and degrade misfolded proteins rather than attempt to re-fold them, so both activities of the protein could be protective in function. Loss of this protective function could be the reason that

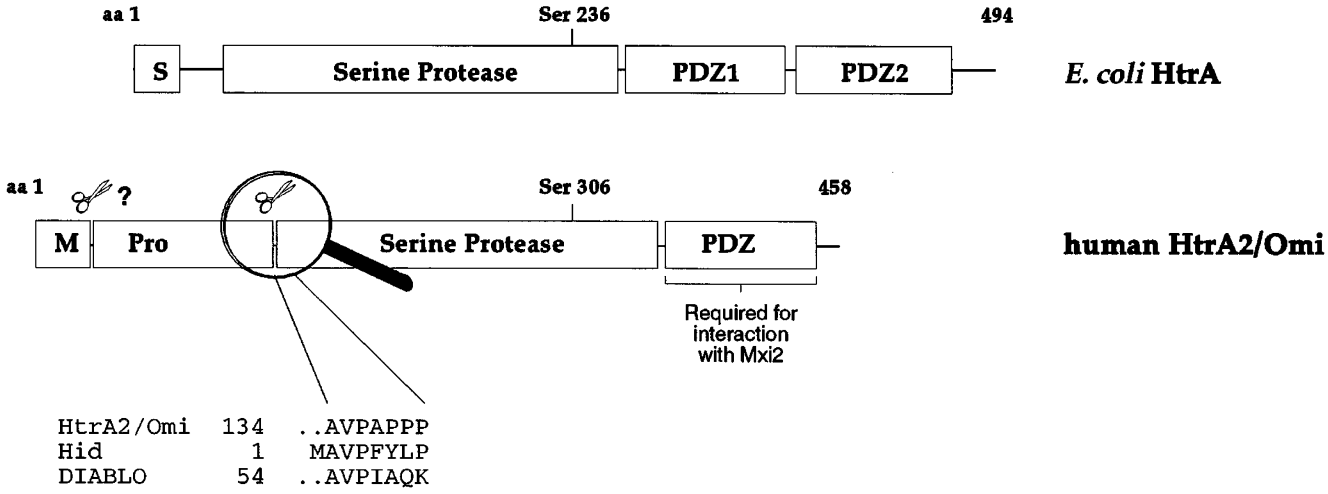

Figure 1 Schematic comparing the protein structure of *E. coli* HtrA and human HtrA2/Omi. *E. coli* HtrA has a potential signal peptide (S), a conserved serine protease domain (Serine Protease), and two PDZ domains. The position of the catalytic serine (Ser) is indicated. Mammalian HtrA2/Omi has a much longer pro domain, with a mitochondrial targetting signal (M) amino acids 1–31, followed by a predicted cleavage site.[4] The mature protein is further processed immediately before amino acid 134 to reveal a conserved IAP binding motif (see line-up below with human Diablo and *Drosophila* Hid). Although there is an apparent autocatalytic requirement for correct processing of HtrA2/Omi it is unknown whether HtrA2/Omi cleaves itself to reveal the IAP binding motif, or whether this processing is due to another mitochondrial protease. The single PDZ of HtrA2/Omi shows highest similarity to the second PDZ of *E. coli* HtrA. PDZ domains are named after three eukaryotic proteins (post-synaptic density protein, disc large and zo-1) and are involved in protein–protein interaction usually by binding to c-terminal tetrapeptides. The function of the PDZ domain in HtrA/HtrA2 is unknown (see review in Pallen and Wren[17]) although the interaction between HtrA2/Omi and Mxi2 requires the PDZ domain of HtrA2/Omi[15]

gram negative pathogens with HtrA mutations are less virulent than their wild-type counterparts, as they would be less able to survive the oxidative attack of their hosts (reviewed in Pallen and Wren[17]). Alternatively, because HtrA processes exported virulence factors, such as Colicin A lysis protein,[18] it is possible that this accounts for their attenuated virulence.

All of this begs the question, what does the multi-functional HtrA2 normally do in the mitochondria of mammalian cells? By analogy to *E. coli* HtrA, HtrA2 is presumably protective in function and is involved in the correct folding/processing and/or proteolytic removal of intermembrane proteins. Like bacterial HtrA, mammalian HtrA2 may also be involved in the processing of specific substrates. Investigating the key substrates of HtrA2 in a healthy mitochondria will provide a rich source of insight into the function of this protein. Other evolutionarily conserved mitochondrial proteins such as cytochrome *c*, endonuclease G and AIF have essential functions in healthy mitochondria but, like HtrA2, can promote cell death in response to apoptotic stimuli. Further studies should reveal whether HtrA2 is just another disorientated mitochondrial escapee or whether it plays specific roles in certain death pathways.

A new and unexpected mammalian regulator of cell death, HtrA2/Omi has stepped out of the shadows. As a protein with ancient evolutionary origins, it probably serves an important housekeeping function in protein re-folding, processing and degradation within mitochondria. Out of harms way in the intermembrane space, the death promoting activities of HtrA2 are masked but, following certain apoptotic insults, HtrA2 is released from the mitochondria into the cytoplasm, where it undergoes a sinister transformation and now actively participates in the demise of the cell.

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