Cell Death and Differentiation (2006) 13, 2007–2010

www.nature.com/cdd

News and Commentary

Mitochondrial membrane remodeling in apoptosis: an inside story

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Cell Death and Differentiation (2006) **13,** 2007–2010.
doi:10.1038/sj.cdd.4402049; published online 13 October 2006

During the early stages of apoptosis, mitochondria are the focal point of a battle for supremacy between opposing factions of the Bcl-2 family. The rival members of this family struggle for control of the permeability of the mitochondrial outer membrane, and ultimately, the life of the cell itself. Should the mitochondrial outer membrane be breached, numerous proteins of the mitochondrial intermembrane space come tumbling out—most notably cytochrome *c*—and this effectively signs the death warrant for the cell. Recent papers from the laboratories of Luca Scorrano and Bart De Strooper[1,2] have shed light upon the mechanism of cytochrome *c* retention within mitochondrial cristae and reveal how remodeling of these structures, to permit efficient redistribution of their contents, is achieved during the early stages of apoptosis.

Cytochrome *c*: Life on the Inside, Death on the Outside

The integrity of the mitochondrial outer membrane has a major impact on cell survival. This is, in large measure, owing to the peculiar fact that cytochrome *c* performs two diametrically opposed roles within the cell. When kept out of harms way within the mitochondrial intermembrane space, cytochrome *c* contributes to maintaining cell viability by performing an important role in the chain of events that results in ATP synthesis. Thus, cytochrome *c* spends the time in its mitochondrial prison keeping out of trouble by shuttling electrons between Complex III (Cytochrome bc_1) and Complex IV (Cytochrome *c* oxidase). However, should the proapoptotic faction of the Bcl-2 family (the 'BH3-only' proteins) manage to conspire with Bax and/or Bak to breach the walls of the mitochondrial penitentiary, there is much trouble in store for the cell. Upon escape into the cytosol, cytochrome *c* wastes no time in making up for its previously benign existence by undergoing a character transformation; much in the same way that the quiet Dr. Jekyll, on occasion, turned into the murderous Mr. Hyde. With cytosolic cytochrome *c* acting as the spur, Apaf-1 and caspase-9 orchestrate a chain of events that leads to widespread proteolytic mayhem and culminates in the death of the cell.[3,4]

Thwarting Escape Attempts

Because of these rather unpleasant consequences, it is clearly important to ensure that cytochrome *c* does not get the opportunity to escape from the confines of the mitochondrial intermembrane space; unless, that is, the intention is to harness its lethal properties and to kill the cell. This is achieved in several ways.

First, several members of the Bcl-2 family are thought to function by maintaining mitochondrial outer membrane integrity and preventing the release of mitochondrial proteins such as cytochrome *c*.[5] Second, even when confined within mitochondria, it seems that most cytochrome *c* is kept within the folds of the inner membrane that are known as cristae.[6] Although previously thought to be open structures that are essentially continuous with the remainder of the intermembrane space, it is now believed that cristae are in fact relatively closed compartments where movement of molecules from within these structures is restricted by the diameter of the openings (i.e. the cristae junctions) at the neck of these structures.[7,8] Thus, even when it is safely locked up within mitochondria, cytochrome *c* is kept within a sort of high security wing where it is restricted from mixing with the other inmates of the intermembrane space (Figure 1).

Third, should a small amount of cytochrome *c* (perhaps the contents of just a few mitochondria) be accidentally released owing to damage or malfunction of a small number of these organelles, this may not be sufficient to promote assembly of Apaf-1/caspase-9 apoptosomes. This view is supported by a recent study from Tang and co-workers, which suggests that the proapoptotic properties of cytochrome *c* can be neutralized by electrostatic interactions with nucleotides, which abound within healthy cells, that prevent this protein from interacting with Apaf-1.[9] Therefore, apoptosis most likely results only when numerous mitochondria become permeabilized to allow the natural barrier to caspase activation posed by high concentrations of endogenous nucleotides to be overcome.

So how does cytochrome *c* manage to breach the barriers that keep this protein under control within a healthy cell? It is now well established that mitochondrial outer membrane permeabilization during apoptosis is largely dependent on the proapoptotic proteins Bax and/or Bak.[10,11] In response to diverse proapoptotic stimuli, these proteins are thought to form a pore or channel – the structure of which remains largely obscure – within the mitochondrial outer membrane that can permit efflux of mitochondrial cytochrome *c*. However, because most cytochrome *c* is sequestered within mitochondrial cristae, it seems that additional factors also impose another level of control upon cytochrome *c* release during apoptosis.

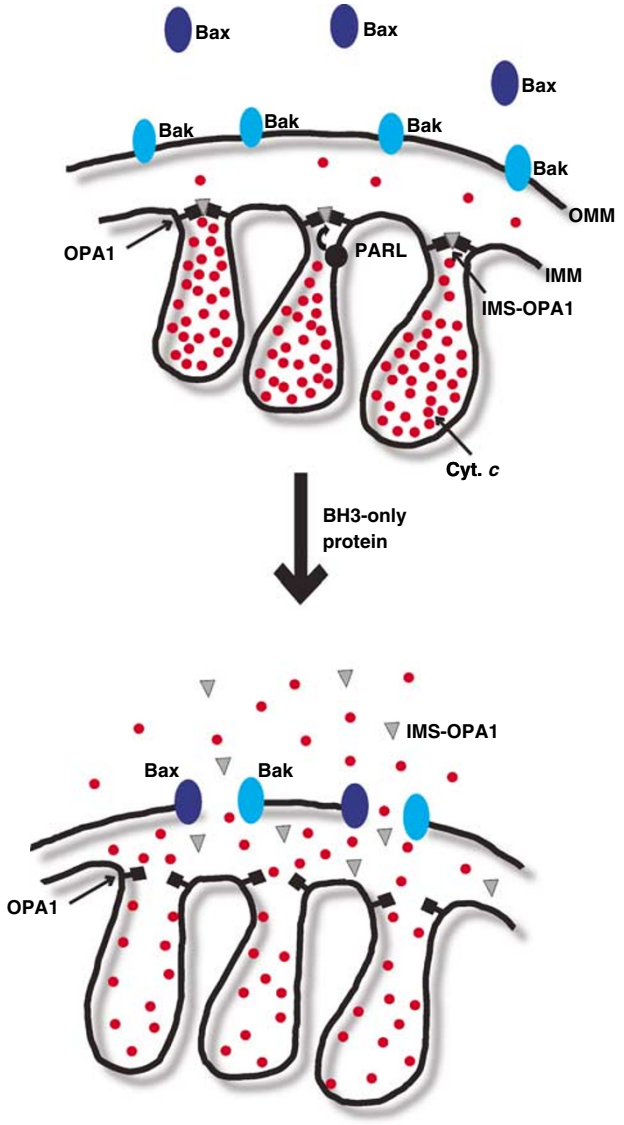

Figure 1 Remodeling the mitochondrial inner membrane during apoptosis. In healthy cells (top), OPA1 oligomers regulate the openings of the cristae junctions and restrict the passage of cytochrome *c*, as well as other cristae proteins, into the mitochondrial intermembrane space (IMS). The inner membrane rhomboid protease, PARL, is required for correct assembly of OPA1 oligomers, possibly by processing a fraction of OPA1 that is found within the intermembrane space as opposed to tethered to the inner membrane. In response to proapoptotic stimuli that activate BH3-only proteins and provoke opening of the Bax/Bak channel (bottom), OPA1 oligomers become destabilized resulting in remodeling of the mitochondrial inner membrane and release of the fraction of cytochrome *c* contained within the cristae. OPA1 may also be released from the intermembrane space during this process. OMM, mitochondrial outer membrane; IMM, mitochondrial inner membrane

OPA1 Regulates Mitochondrial Cristae Integrity

In addition to Bax/Bak-dependent permeabilization of the mitochondrial outer membrane, extensive remodeling of the mitochondrial inner membrane also appears to be required for efficient redistribution of cytochrome *c* to the cytosol.[6] This is because as much as 90% of the mitochondrial cytochrome *c* content may be sequestered within the cristae folds of the mitochondrial inner membrane. These folds appear to act as a sub-compartment of the intermembrane space and, because of the restricted space at the neck of these folds, can restrict free passage of their contents out of mitochondria even if the outer membrane is breached.

Frezza *et al.*[1] now provide evidence that OPA1 is involved in maintaining the integrity of cristae junctions by forming oligomeric complexes between inner membrane-localized OPA1 molecules and shorter forms of the same protein that are found within the intermembrane space. Consistent with this, overexpression of OPA1 delayed the release of mitochondrial cytochrome *c* and apoptosis in response to exposure of mouse embryonic fibroblasts (MEFs) to stimuli that activate the intrinsic pathway to apoptosis.[1] Cells overexpressing OPA1 displayed remodeled cristae networks with somewhat narrower cristae junctions that resisted Bid-induced opening more efficiently than cristae from wild-type mitochondria. Conversely, RNAi-mediated ablation of OPA1 resulted in more rapid release of mitochondrial cytochrome *c* and such cells underwent apoptosis more readily than controls.[1]

Previous studies on transformed cell lines have also found that RNAi-mediated ablation of OPA1 results in extensive reorganization of mitochondrial cristae, fragmentation of the mitochondrial network and a dramatic increase in spontaneous apoptosis.[12–14] Such cells are also hypersensitive to diverse proapoptotic stimuli.[1,13] Moreover, Arnoult *et al.*[14] have also shown that OPA1 is one of the many proteins that become released from the mitochondrial intermembrane space during apoptosis. Because OPA1 appears to be required for the integrity of mitochondrial cristae and also for normal mitochondrial morphology, this raises the possibility that apoptosis-associated release of OPA1 into the cytosol underpins the extensive fragmentation of the mitochondrial network that is frequently observed during this mode of cell death.[15–17] Whereas the latter proposal remains speculative, it does seem that OPA1 serves an indispensable role in organizing mitochondrial cristae into their correct form and that interference with the function of this protein results in an increased potential for cytochrome *c* escape.

PARL is Required for OPA1 Function

In an accompanying paper in the same issue of *Cell*, Cipolat *et al.*[2] provide evidence that the mitochondrial inner membrane rhomboid protease, PARL, may be required for the correct assembly of the OPA1-containing structures that regulate the integrity of the cristae junctions. Both PARL and OPA1 are associated with the mitochondrial inner membrane and there is some evidence that these proteins can interact.[2] Because the yeast homolog of OPA1 (Mgm1p) is a substrate for the yeast rhomboid protease Rdb1p,[18] it is tempting to assume that PARL may proteolytically process OPA1. However, significant doubt surrounds whether PARL can directly process OPA1.[19] Nevertheless, this protease does seem to be required for the production of a small fraction (~4%) of OPA1 – perhaps through processing of a downstream protease – that is found free within the mitochondrial

intermembrane space in healthy cells.[2] This small pool of OPA1 displays a faster mobility on SDS-PAGE than membrane-bound OPA1, suggestive of proteolytic processing, and may be critical for maintaining the integrity of the cristae junctions.[2] Cipolat *et al.*[2] propose that these shorter forms of OPA1 may bridge the space between membrane-bound OPA1 molecules at the cristae junctions (Figure 1).

Although PARL may not be responsible for proteolytic-processing of OPA1 directly, loss of PARL through gene targeting resulted in a reduction in the fraction of OPA1 that is normally found within the intermembrane space.[2] PARL-deficient mitochondria more readily released cytochrome *c* upon challenge with proapoptotic stimuli or treatment with recombinant Bid.[2] Although overexpression of OPA1 in wild-type MEFs delayed cytochrome *c* release and apoptosis, this effect was not seen in MEFs derived from PARL null animals.[1] Significantly, the latter result suggests that the antiapoptotic effects of OPA1 require PARL. In line with this, MEFs from PARL null mice underwent apoptosis more readily upon exposure to a variety of stimuli that engage the mitochondrial pathway to apoptosis. Although mitochondria isolated from these animals displayed apparently normal mitochondrial function, increased rates of apoptosis were observed in a variety of tissues in these animals, including thymus and spleen. PARL null animals were born at Mendelian ratios and failed to display any obvious deficiencies in rates of developmental apoptosis. However, these mice died within 12 weeks of birth, apparently owing to extensive muscle wasting and weight loss. It is suggested by the authors that the underlying defect contributing to the death of these mice is increased rates of apoptosis within the affected tissues owing to the normal requirement for PARL in maintaining cristae integrity via processing of OPA1.[2] However, although this seems plausible, other explanations are also clearly possible.

Mitochondrial Fission and Apoptosis

It should be noted that the events which remodel mitochondrial inner membranes during apoptosis appear to be separable from those that result in mitochondrial fragmentation, as the loss of PARL did not have any impact upon mitochondrial fission or fusion dynamics.[2] Although it is clear that extensive mitochondrial fission is associated with apoptosis,[15–17] it remains a matter of debate as to whether this phenomenon also contributes to cytochrome *c* efflux. Although initial studies from Youle's laboratory suggested that interfering with the process of mitochondrial fission could delay the release of cytochrome *c* and consequent apoptosis,[20] subsequent reports suggest that these events may be coincident rather than functionally interconnected.[14,21] Moreover, data from Arnoult *et al.*[14] suggest that cytochrome *c* release may even occur before any discernable fragmentation of the mitochondrial network. At present, the preponderance of evidence suggests that although Bax and/or Bak activation within the outer mitochondrial membrane can undoubtedly provoke collapse and fragmentation of normal mitochondrial networks, this may not be strictly required for cytochrome *c* release. It is also clear that mitochondrial networks can become extensively fragmented without releasing intermembrane space proteins or provoking apoptosis.[13,21]

It has also been proposed that some members of the Bcl-2 family, as a secondary function to their role in regulating apoptosis, may also contribute to mitochondrial fusion and fission dynamics in healthy cells.[21] Interference with the latter function (as a consequence of interaction with proapoptotic Bcl-2 family members) may therefore result in collapse of the mitochondrial network in parallel with the formation of Bax/Bak oligomers that permit escape of proteins from the mitochondrial intermembrane space.

Letting the Genie out of the Bottle

Taking these observations together, Scorrano and De Strooper[1,2] have proposed a model whereby OPA1 maintains the tightness of the mitochondrial cristae junctions through the formation of oligomers between membrane-bound OPA1 isoforms and shorter OPA1 isoforms located within the intermembrane space. PARL seems to be required for the proper formation of these OPA1 oligomers via processing of OPA1, either directly, or indirectly. Thus, OPA1 contributes to the maintenance of normal mitochondrial cristae where much of the cell's arsenal of cytochrome *c* is stored. During apoptosis, OPA1 oligomers may become destabilized, either owing to the release of the soluble intermembrane space fraction of OPA1 upon opening of the Bax/Bak channel in the mitochondrial outer membrane, or via some other mechanism. The latter event results in a dramatic weakening of the cristae junctions and consequent release of their contents (Figure 1).

Although this scenario is indeed attractive, some questions remain unresolved. It is unclear precisely what role PARL plays in all of this as OPA1 may not be a direct substrate for this protease.[19] Similarly, the composition of the OPA1 complex at the cristae junctions requires further definition, as does the intermembrane space form of this protein. How OPA1 complexes become destabilized upon Bax/Bak activation within the outer mitochondrial membrane is obscure. Furthermore, although OPA1 does appear to delay the release of cytochrome *c* by maintaining the integrity of mitochondrial cristae, it seems unlikely that this is a major checkpoint in apoptosis as the protection afforded by OPA1 overexpression is modest as compared with overexpression of Bcl-2 or Bcl-x_L, for example.

These quibbles notwithstanding, the recent papers from Scorrano and De Strooper contribute to our understanding of the mechanism of cytochrome *c* release during apoptosis and further underscore the importance of this organelle within the mammalian cell death machinery.

Acknowledgements

We are indebted to Science Foundation Ireland for their support of ongoing work in our laboratory. SJM is a Science Foundation Ireland Principal Investigator.

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