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Review

Mitochondrial fission and apoptosis: An ongoing trial

Philippe A. Parone, Jean-Claude Martinou *

Department of Cell Biology, University of Geneva, Quai Ernest-Ansermet 30, 1211 Geneva 4, Switzerland

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Abstract

Apoptosis is a form of programmed cell death that is essential for the development and tissue homeostasis in all metazoan animals. Mitochondria play a critical role during apoptosis, since the release of pro-apoptogenic proteins from the organelle is a pivotal event in cell death triggered by many cytotoxic stimuli. A striking morphological change occurring during apoptosis is the disintegration of the semi-reticular mitochondrial network into small punctiform organelles. It is only recently that this event has been shown to require the activity of proteins involved in the physiological processes of mitochondrial fission and fusion. Here, we discuss how this mitochondrial morphological transition occurs during cell death and the role that it may have in apoptosis.

Keywords: Mitochondria; Apoptosis; Fission; Fusion; Cytochrome c; Mitochondrial membrane permeabilisation

1. Introduction

Apoptosis is a form of programmed cell death that is essential for the development of the embryo and to maintain tissue homeostasis in adult multicellular organisms. Disrupting such a vital process leads to a range of diseases, such as cancer, neurodegeneration and autoimmunity. Apoptosis is characterized by a stereotyped series of morphological changes including membrane blebbing, cell shrinkage, chromatin condensation and inter-nucleosomal DNA cleavage. This process culminates in the formation of apoptotic bodies, plasma membrane vesicles enclosing the digested cellular material, which are engulfed by neighbouring cells and macrophages, thus preventing inflammation [1,2]. This orderly destruction of the cell is orchestrated by a family of cysteine proteases called caspases [3]. Given their pre-eminent role in apoptosis, it is reasonable to suppose that caspases are strictly controlled. Depending on the cell type and death stimulus, two pathways are known to regulate caspases: (1) the extrinsic pathway, which is initiated by the binding of a death ligand to its death receptor resulting in the rapid activation of caspases [4]; (2) the intrinsic pathway, whereby the death stimulus activates a signalling cascade triggering mitochondrial outer membrane permeabilisation (MOMP) and the release of pro-apoptogenic factors into the cytosol [5,6]. Some of these proteins are now known to contribute to the activation of caspases either through the apoptosome (cytochrome *c*) or by binding to IAPs (Inhibitors of Apoptosis Proteins; Smac/Diablo, HtrA2/Omi) [7]. MOMP is a critical event in the intrinsic apoptotic pathway and it is induced by a subclass of pro-apoptotic Bcl-2 proteins which includes Bax and Bak [8]. These proteins seem to be in an inactive state in healthy cells, with Bax mostly found in the cytosol. However, during apoptosis induced by various death stimuli, including DNA damage or trophic factor deprivation, they are activated by a process requiring BH3-only Bcl-2 family members [9,10]. It is thought that BH3-only proteins bind and sequester Bcl-2 anti-apoptotic proteins (this is the case for Bad and NOXA), or bind to and directly activate pro-apoptotic proteins (tBid for example). This leads to the inactivation of Bcl-2 anti-apoptotic proteins and the oligomerisation/insertion of Bax and Bak in the MOM, which results in MOMP.

The mechanisms underlying MOMP are still unclear and are the subject of much debate [9,11]. A new twist to the story was brought on by the rediscovery that mitochondria fragment during cell death, an event that has recently been shown to require proteins of the mitochondrial fission machinery [12]. The scope of this review is to summarise these findings and

* Corresponding author. Tel.: +41 22 702 64 43; fax: +41 22 702 64 42.
E-mail address: Jean-Claude.Martinou@cellbio.unige.ch (J.-C. Martinou).

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address three fundamental questions concerning the physiological relevance of mitochondrial fission in apoptosis: (1) how is fission activated during cell death? (2) What role does fission of the mitochondria play in MOMP? (3) Is mitochondrial fission required for cell death?

2. Mitochondria fragment during apoptosis

The mitochondria form a highly dynamic semi-tubular network, the morphology of which is regulated by movements along the cytoskeleton and frequent fission and fusion events. Our understanding of the mechanisms underlying mitochondrial fission and fusion mostly comes from studies in yeast. Coincidentally, the molecular components implicated in both these processes are highly conserved in other species such as mammals, *C. elegans* and *Drosophila* [13,14]. For the purpose of this review only a brief outline of the proteins involved in mitochondrial fission and fusion in mammalian cells will be given. At least four proteins, Drp1, hFis1, MTP18 [15,16] and GDAP1 [17,18] are required for mitochondrial fission. The dynamin-related protein Drp1 is a large cytosolic GTPase that translocates to the mitochondria where it couples GTP hydrolysis with scission of the mitochondrial tubule [19–21]. Its receptor at the surface of the mitochondria is thought to be hFis1, which is anchored to the MOM facing the cytoplasm [22–25]. Mitochondrial fusion in mammalian cells utilises a different set of proteins: the large transmembrane GTPase mitofusins (Mfn1, Mfn2) anchored on the MOM and the dynamin-like GTPase OPA1 located in the mitochondrial intermembrane space [13,14,26].

A striking event occurring during apoptosis is the disintegration of the semi-reticular mitochondrial network into small punctiform organelles. Initially, before mitochondria could be observed in live cells, ultra-structural studies showed that in apoptotic cells the mitochondria were structurally intact but appeared to be condensed [27–31]. A reduction of the mitochondrial volume had already been observed in pathological tissue, which were retrospectively found to undergo apoptosis [32–35]. More recently, ultra-condensation or fragmentation of mitochondria was observed in a range of cell types undergoing apoptosis [36–38]. Interestingly, this change in mitochondrial morphology is reversible in sympathetic neurons upon re-addition of the trophic factor to the culture medium [39]. Of note in addition to the mitochondria becoming fragmented during apoptosis, the organelles, which are normally evenly distributed in healthy cells, cluster around the nucleus during cell death. This relocation of the mitochondria has been attributed to the inactivation of mitochondria-associated kinesin [40].

Since mitochondrial condensation or fragmentation has been observed so consistently during apoptosis, this begs the question as to what is the molecular mechanism regulating this process. Frank and co-workers were the first to address this question and hypothesized that components of the "classical" mitochondrial fission machinery were involved in the fragmentation of the mitochondria during apoptosis. The authors show that Drp1 translocates to the mitochondria during Bax/Bak-dependent apoptosis and that inhibiting the activity of Drp1 (by over-expression of the dominant negative mutant DrpK38A) efficiently prevents fragmentation of the mitochondria [12]. These results were supported by a more recent study from the same laboratory showing that down-regulating the expression of hFis1 or Drp1 prevents fragmentation of the mitochondrial network during Bax/Bak-dependent apoptosis. Drp1-dependent fragmentation of the mitochondrial network has also been observed in a different death paradigm. Work from Breckenridge and co-workers [41] implicated mitochondrial fission in the fragmentation of the organelle during death induced by p20, the product of BAP31 caspase cleavage during Fas mediated death. In this study the authors show that the increase in cytosolic calcium triggered by p20 expression is required for mitochondrial fragmentation, which occurs after the recruitment of Drp1 to the organelle, and that disintegration of the mitochondrial network is inhibited by the expression DrpK38A [41]. In an effort to further elucidate the molecular mechanisms regulating the fragmentation of the mitochondrial network during apoptosis, Sugioka and colleagues [47] inhibited this event by activating the mitochondrial fusion machinery. The authors show that over-expression of rat Fzo1A, B (homologues of the human mitofusins) in HeLa cells prevents the fragmentation of the mitochondrial network during Bax/Bak-dependent cell death, thus suggesting that mitochondrial fusion might be affected during apoptosis. This is in agreement with work from Karbowski and co-workers [42,45] who used a mitochondrially targeted photoactivable green fluorescent protein to assess the rates of mitochondrial fusion during apoptosis. The authors show that the diffusion rate of the probe after photoactivation is significantly decreased in cells undergoing apoptosis, thus suggesting that mitochondrial fusion is inhibited during cell death [42].

Altogether these studies show that mitochondrial fragmentation occurring during apoptosis is mediated by an increase in the fission of the organelle, following the recruitment of Drp1, and an inhibition of fusion. How are these two events regulated during apoptosis? It has been proposed that Bax plays a critical role in this control. This is based on the finding that Bax co-localizes with Drp1 and Mfn2 in distinct foci, possibly future scission sites, on the mitochondria after the induction of apoptosis [43,44]. Furthermore, endophilin B1, a protein required for the maintenance of the mitochondrial morphology and that translocates to the mitochondria during cell death [45], has been shown to interact with Bax only after the induction of apoptosis [46]. These results would suggest that Bax regulates the morphological changes of the mitochondria during apoptosis through its interaction with components of the mitochondrial fission and fusion machinery. However, there is no evidence in the literature supporting the existence of a direct interaction between Bax and Drp1 or Mfn2. Nevertheless, such interactions might exist but may be transient and difficult to detect. A second concern about this model is that Bcl-2, a well-established inhibitor of Bax, does not prevent mitochondrial fission [47]. Furthermore, Drp1 is equally recruited to mitochondria during apoptosis in cells lacking Bax and Bak [47]. However, we cannot exclude that Bcl-2 is not able to

inhibit the activity of Bax as a regulator of mitochondrial morphological changes and that Drp1 translocation does not depend on Bax/Bak. Interestingly, over-expression of tBid (corresponding to the caspase cleaved active form of Bid), a known activator of Bax [48], does not lead to recruitment of Drp1 or fragmentation of the mitochondrial network during apoptosis [47]. Attention should also be drawn to the fact that the co-localization of Bax with Mfn2 in distinct foci is in disagreement with previous studies showing that Mfn2 distributes evenly on the mitochondria without forming visible foci [49–52]. However, it remains possible that Mfn2 aggregates in distinct regions of the mitochondria only after the induction of apoptosis, although this has not been shown yet. Finally, it is unlikely that Bax activates the mitochondrial fission machinery after ER calcium release following p20 over-expression, since in this case mitochondrial fragmentation precedes the activation of Bax and the release of cytochrome *c* [41].

Recent evidence has been published that provides an alternative mechanism to explain how mitochondrial fission is activated and fusion is inhibited during cell death. Detailed kinetic studies have revealed that mitochondria fragment after the release of cytochrome *c* in mammalian cells and in trypanosoma [53–55]. Incidentally, Arnoult and co-workers find that OPA1 and DDP/TIMM8a are both released from the mitochondria after MOMP during Bax/Bak-dependent cell death [53,56]. The release of OPA1 could potentially explain the inhibition of mitochondrial fusion occurring during apoptosis, since mitochondria in OPA1 depleted cells are heavily fragmented [57–59]. Concerning DDP/TIMM8a, the authors show that it binds to Drp1 and promotes its recruitment to the mitochondria, thus providing a mechanism for how mitochondrial fission is activated during apoptosis. Nevertheless, this model does not explain why Bcl-2 over-expression is unable to prevent mitochondrial fragmentation during Bax/Bak-dependent cell death [47]. Of note, the data from Arnoult and co-workers does not exclude that in other death paradigms, such as in death receptor activated apoptosis, mitochondrial fragmentation might occur prior to the activation of Bax and cytochrome *c* release, as shown by Breckenridge and colleagues [41].

In summary, mitochondrial fragmentation occurs in most forms of apoptosis and is due to activation of mitochondrial fission machinery as well as the inhibition of fusion. However, how this process is regulated is still unclear (see Fig. 1).

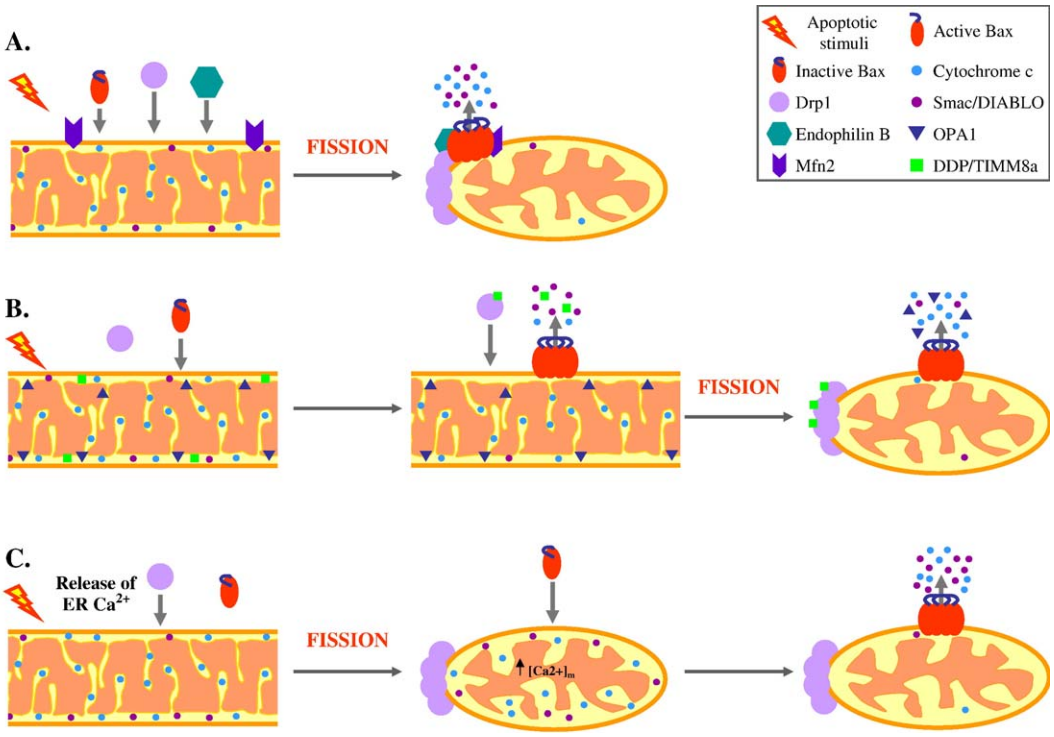

Fig. 1. Model mechanisms for the activation of mitochondrial fission during cell death and for the role of fission in MOMP. (A) Bax/Bak-dependent death stimuli trigger the translocation of Bax, Drp1 and Endophilin B1 to the mitochondria. Active Bax participates in the activation of Drp1-dependent fission of the mitochondria and also inhibits fusion of the organelle through its interaction with Mfn2. Remodelling of the inner mitochondrial space occurs by an unknown mechanism possibly linked to the activation of fission or inhibition of fusion. Activated Bax forms pores across the MOM that contribute to MOMP. (B) Bax/Bak-dependent death stimuli trigger the translocation of Bax to the mitochondria. Active Bax forms oligomers that allow the release of proteins soluble in the inter membrane space of the mitochondria, such as Smac/DIABLO, DDP/TIMM8a and some cytochrome *c*. Once in the cytosol, DDP/TIMM8a binds to Drp1 and promotes its translocation to the mitochondria. This event is followed by Drp1-dependent fission, release of OPA1, remodeling of the intramitochondrial space due to the loss of OPA1 and release of the remaining cytochrome *c*. (C) Pro-apoptotic signals triggering the release of ER calcium, such as p20 over-expression, lead to translocation of Drp1 to the mitochondria and fission of the organelle. Remodeling of the intra-mitochondrial space occurs through a Drp1-dependent mechanism and possibly also due to the rise in mitochondrial calcium. This is followed by the translocation of Bax and the formation of Bax containing pores that trigger the release of pro-apoptotic proteins from the mitochondria.

3. Mitochondrial fission and the release of apoptogenic proteins

It is now becoming accepted that mitochondrial fragmentation is an integral part of most forms of apoptosis. However, the role of this change in mitochondrial morphology during cell death is still unclear. As mentioned above, disintegration of the mitochondrial network occurs within the same time frame as Bax activation and MOMP in most cases studied. Although the precise mechanisms of MOMP are still debated, it is accepted that Bax and Bak are essential for this process [60]. One model, based on the structural similarity of Bax/Bak to certain pore forming bacterial toxins, suggests that these pro-apoptotic Bcl-2 proteins form large channels that permeabilise the MOM [61,62]. An alternative model proposes that MOMP occurs following the formation of a large conductance channel containing Bax and components of the permeability transition pore (PTP), such as the voltage-dependent anion-selective channel (VDAC; [63]) and the adenine nucleotide translocator (ANT; [64]). Does mitochondrial fission play a role in MOMP? Several studies have reported that preventing mitochondrial fission during apoptosis leads to a partial inhibition of cytochrome *c* release [12,41,44,47,59]. This suggests that mitochondrial fragmentation or components of the mitochondrial fission/fusion machinery could be involved in one of the events leading to MOMP, such as the translocation and/or the activation of Bax/Bak at the MOM. The translocation of Bax and its activation at the mitochondria have been shown to occur normally in cells depleted of Drp1 or transfected with DrpK38A [12,43,59,65]. This is in disagreement with results obtained by Neuspiel and co-workers [44] who show that the amount of conformationally active Bax at the mitochondria is significantly reduced in DrpK38A transfected cells [44]. Furthermore, over-expression of rat Fzo1A,B also delays the translocation of Bax to the mitochondria and the oligomerisation of Bax and Bak at the MOM [47]. Nevertheless, preventing mitochondrial fission during apoptosis by depleting cells of hFis1 instead of Drp1 does prevent Bax translocation and activation at the mitochondria [59]. Altogether, it is still not completely clear if and how preventing mitochondrial fragmentation during cell death affects the translocation of Bax and the activation of Bax/Bak at the MOM. It is possible that different components of the mitochondrial fission/fusion machinery are required at distinct steps of the activation of Bax/Bak. The fused morphology of the mitochondria in hFis1-depleted cells or in cells over-expressing Fzo1A,B might hamper the insertion of Bax/Bak in the MOM. This would be consistent with previous data showing that membrane curvature influences the permeabilisation of liposomes by Bax [66,67]. However, inhibiting the function of Drp1 also leads to fusion of the mitochondrial network and according to several studies activation of Bax is not inhibited in these cells. As mentioned previously, Bax has been shown to form clusters at scission sites together with Drp1 and Mfn2, possibly implying that either of these two proteins could be involved in the activation or the targeting of Bax to the mitochondria. Nevertheless, in addition to the concerns raised above regarding this hypothesis, it has been proposed that the appearance of the Bax, Drp1, Mfn2 containing clusters follows cytochrome *c* release and thus might be subsequent to an initial activation of Bax [53]. Yet this does not exclude that components of the fission/fusion machinery or the fragmentation of mitochondria per se, might be required to further recruit and/or activate Bax/Bak after an initial permeabilisation of the MOM. In this scenario, fragmentation of the mitochondria during apoptosis could be a positive feedback mechanism to amplify MOMP. Nevertheless, mitochondrial fragmentation is not always required for MOMP. Very limited mitochondrial fragmentation occurs in mouse embryonic fibroblasts during etoposide induced cell death and no mitochondrial fission was observed when cell death was induced by tBid over-expression [47]. Furthermore, MOMP has been shown to occur in the extensively fused mitochondria of MTP18 depleted cells [15]. Finally, cytochrome *c* release has been shown to occur with the same kinetics at 37 °C or 24 °C. This, therefore, excludes the involvement of the GTPase enzymatic activity of Drp1, or other GTPases involved in mitochondrial fission and fusion, in the release of cytochrome *c*.

Until recently, it was thought that MOMP was sufficient for the complete release of cytochrome *c*. However, two pools of cytochrome *c* exist within the mitochondria: a minor pool that is soluble in the intermembrane space (IMS) and a major pool confined to the mitochondrial cisternae [68–70]. Furthermore, electron microscopic tomography of mitochondria revealed that the IMS is separated from the intra-cisternal space by narrow cristae junctions [71]. Therefore, to account for the complete and fast release of cytochrome *c* during apoptosis [72], cisternal cytochrome *c* must be relocated into the IMS before it can be released through the Bax/Bak containing pore in the MOM. Recently, it has been shown that several apoptotic stimuli cause disruption of cristae junctions, resulting in opening of the cristae and in the expansion of the intermembrane space [68,73,74]. In particular, Scorrano and co-workers [68] showed that tBid not only activates Bax/Bak to permeabilise the MOM but also independently mediates a remodeling of the mitochondrial cristae which is required for the complete release of cytochrome *c* from the mitochondria. This intra-mitochondrial remodeling, specified as Class II by the authors, is characterized by a network of fused cristae with widened inter-cristae junctions. What is the role of mitochondrial fission and fusion in the intra-mitochondrial rearrangements occurring during apoptosis? The molecular mechanisms regulating cristae morphology and architecture are still unclear. Nevertheless, mitochondrial proteins required to maintain normal mitochondrial shape have been implicated in this process by recent studies. The yeast MIM protein Mdm33 and the MOM protein Mmm1p have been shown to play an important role in intra-mitochondrial organization [75,76]. In mammalian cells, mitofilin, an IMS protein, is required for proper formation of MIM cristae [77]. Nevertheless, the intra-mitochondrial rearrangements occurring following the inactivation of the proteins listed above are not similar to those in the Class II mitochondria described by Scorrano and colleagues [68]. In contrast, the cristae morphology of mitochondria from OPA1 depleted cells is highly reminiscent of that in Class II mitochondria [53,57,58].

Furthermore, down-regulating the expression of OPA1 sensitizes mammalian cells to apoptosis [58,59] and mitochondria isolated from these cells release cytochrome *c* more readily [53]. Finally, as mentioned above, OPA1 is released into the cytosol during apoptosis induced by Bax/Bak-dependent death stimuli [53]. These results suggest that OPA1 is likely to play an important role in the remodeling of the intra-mitochondrial space during apoptosis.

Drp1 has also been proposed to regulate intra-mitochondrial rearrangements during apoptosis. This is based on the finding that dominant negative DrpK38E inhibits remodeling of the cristae following BIK induced apoptosis [65]. How Drp1, a MOM protein, is involved in this process is still unclear. Other dynamins, including dynamins 1 and 2, have previously been reported to have activities independent of their role in membrane fission due to, for example, their ability to bind other proteins. One such protein is endophilin A1, which has been shown to affect membrane curvature [78,79]. Interestingly, endophilin B1 is a homologue of this protein [80,81]. Whether Drp1 recruits or synergizes with endophilin B1 to remodel the MOM and the intra-mitochondrial space still remains to be demonstrated. Alternatively, dynamins have also been shown to modulate actin dynamics at the membrane [82,83] or initiate intracellular signaling cascades [84,85], both of which could potentially explain how Drp1 induces remodeling of the MIM. In summary, certain studies suggest that proteins involved in mitochondrial fission/fusion may be implicated in the release of pro-apoptotic factors from the mitochondria (Fig. 1). However, it is not yet clear if these proteins are absolutely required for this process and what role they play in it. Nevertheless, in line with the hypothesis presented above, namely that mitochondrial fragmentation could act as a positive feedback mechanism to amplify Bax/Bak activation, fission of the organelle during apoptosis could be involved in the remodeling of the intra-mitochondrial space and therefore amplify the release of cytochrome *c* by liberating the protein from the cisternal pool.

4. Mitochondrial fission and cell death

The release of pro-apoptogenic proteins from the mitochondria is a critical event in cell death mediated through the intrinsic apoptotic pathway [11]. Since mitochondrial fragmentation may be involved in this process, is it sufficient to inhibit mitochondrial fission in order to prevent cell death? To this date a number of studies have addressed this question. Inhibiting mitochondrial fission by over-expressing DrpK38A has been suggested to inhibit apoptosis [12]. However, this result should be interpreted cautiously. In fact, the authors compared the rate of apoptosis of DrpK38A cells to that of DrpWT transfectants, and it has been shown since that over-expression of DrpWT sensitizes cells to death induced by staurosporine for example [43,86]. This suggests that the protection that over-expression of DrpK38A confers to cells might have been overestimated in the Frank et al. study [12]. Consistently, depleting cells of Drp1 only confers mild protection against cell death induced by a range of cytotoxic stimuli [59]. However, in the same study, the authors find that depleting cells of hFis1 has a strong anti-apoptotic effect under the same conditions [59]. Although from these studies, it is difficult to conclude that preventing mitochondrial fission inhibits cell death, since apoptosis was assessed at single time points, it cannot be excluded that depleting cells of Drp1 or hFis1 delays the progression of apoptosis. In agreement with this suggestion, Drp1 depletion or Fzo1A,B over-expression delays death induced by a number of apoptotic stimuli as assessed in time course experiments [47]. Whereas apoptosis induced by cytotoxic stimuli, such as staurosporine or etoposide, is delayed when mitochondrial fission is inhibited, calcium mediated cell death is partially prevented when mitochondrial fragmentation is enhanced by the over-expression of DrpWT [86]. This result does not concur with data showing that DrpK38A partially inhibits cytochrome *c* release and caspase 3 activation following release of ER calcium induced by over-expression of p20 [41].

An alternative role for mitochondrial fission during programmed cell death (PCD) has recently been proposed. Studies have shown that mitochondria are eliminated during PCD through a caspase-independent process known as mitoptosis [87–89]. Arnoult and co-workers now provide evidence that Drp1 mediated fission of the mitochondrial network promotes the elimination of the mitochondria during cell death, whereas expressing DrpK38A retards the disappearance of the organelle [56]. These results suggest that mitochondrial fission might play an active role in an alternative form of PCD known as autophagic cell death [90,91].

Inducing mitochondrial fragmentation by over-expression of hFis1 or down-regulation of OPA1 has been shown to cause the release of cytochrome *c* and cell death in the absence of apoptotic stimuli [25,58]. Excessive mitochondrial fragmentation induced by the over-expression of DrpWT in *C. elegans* has also been shown to induce cell death [92]. These results have often been used as evidence to suggest that mitochondrial fission is required for cell death. However, this is probably an over ambitious conclusion since there are many examples of enhanced mitochondrial fission, or impaired mitochondrial fusion, that do not result in cell death. Mammalian cells treated with protonophores, which dissipate the MIM potential thus inhibiting fusion, display heavily fragmented mitochondria but remain viable for up to 24 h and their mitochondria retain cytochrome *c* [93–95]. Healthy embryonic fibroblasts from knockout mice lacking Mfn1 or Mfn2 can survive despite having profoundly fragmented mitochondria [50]. A range of cell types in which the expression of Fzo1A,B was down-regulated do not die in the absence of additional apoptotic stimuli [47]. The anti-apoptotic factor vMIA encoded by cytomegalovirus induces mitochondrial fragmentation but inhibits cytochrome *c* release and cell death [96]. Finally, over-expression of DrpWT leads to fission of the mitochondrial network without inducing cell death; on the contrary these cells are protected against apoptosis induced by ceramide [86]. In view of the results presented here, it would be more cautious to suggest that apoptosis induced in certain cases of excessive fission could be due to signals other than those regulating mitochondrial fragmentation. Alternatively, it is possible that mitochondria from OPA1 or hFis1 depleted cells are damaged

and that this is the cause of death. In support of this hypothesis, mitochondrial dysfunction has previously been shown to trigger apoptosis [97–100]. Furthermore, OPA1 depleted cells have severely decreased cellular respiration [101]. Finally, in patients with OPA1 mutations, suffering from autosomal-dominant optic atrophy (ADOA), the copy number of mitochondrial DNA (mtDNA) molecules is lower and oxidative phosphorylation is decreased in the calf muscle of these patients [102,103].

The role of mitochondrial fission in apoptosis has also been studied in eukaryotes other than mammals. Studies in yeast have shown that treating the cells with acetic acid or H_2O_2 leads to fragmentation of the mitochondrial network and programmed cell death [104]. Deleting the Drp1 homologue in yeast (Dnm1) protected as much as 30% of the cells against apoptosis. Interestingly, however, Dnm1-deficiency protected from cell death more efficiently than from mitochondrial fragmentation. This suggests that the absence of Dnm1 in yeast might confer protection against cell death also by mechanisms other than those related to fission of the mitochondria. In contrast to the results obtained in mammalian cells, deletion of Fis in yeast sensitizes the cells to apoptosis and leads to an increase in cell death of approximately 30% [104]. In *C. elegans*, Drp1-dependent mitochondrial fragmentation is induced by the BH3-only protein EGL-1 in cells undergoing PCD during development [92]. However, when mitochondrial fission is blocked, by the expression of DrpK40A, 90% of the cells destined to die during development do undergo PCD as planned. Moreover, ced-9(n1950gf) and ced-9(n2812lf) both blocked mitochondrial fragmentation induced by EGL-1 or Drp1 but ced-9(n2812lf) animals, contrary to ced-9(n1950gf) animals, are not viable as a result of ectopic programmed cell death. This implies that even when mitochondrial fission is inhibited, PCD still occurs during development. These results suggest that mitochondrial fission is not required for cell death. However, this does not exclude that fragmentation of the mitochondrial network might potentiate cell death, as suggested by the authors.

In summary, it is not yet clear if mitochondrial fragmentation is required for apoptosis. However, it is possible that preventing mitochondrial morphological changes might delay apoptosis without inhibiting it. The delay could be attributed to the partial inhibition of cytochrome *c* release in these cells. Nevertheless, we cannot exclude that the tools we use to prevent mitochondrial fission during apoptosis also lead to changes in mitochondrial function, thus possibly affecting the rate of apoptosis. Therefore, results obtained using such tools should be interpreted more carefully, and all possible reasons should be examined or at least considered.

5. Concluding remarks

Over the past few years, mitochondrial fission has been shown to occur in a range of cell types after the induction of apoptosis by different death stimuli. Certain studies would suggest that pro-apoptotic Bcl-2 family members are involved in activating mitochondrial fragmentation during apoptosis, but the mechanism involved remains elusive. Moreover, several lines of evidence argue against the possibility that Bax or Bak might activate changes in mitochondrial morphology during cell death. Mitochondrial fission has been shown to occur before and after MOMP, depending on the death stimuli. Therefore, it is not yet clear if mitochondrial fragmentation is absolutely required for the permeabilisation of the MOM. Nevertheless, it might provide a positive feedback mechanism to amplify the initial release of pro-apoptogenic proteins from the mitochondria. This would explain why a number of studies have found that preventing mitochondrial fission delays cell death. However, this is probably a simplistic conclusion since the effects that preventing mitochondrial fission has on the function of the organelle and on cellular homeostasis have never been considered. Yet, this does not exclude that mitochondrial fission might be required for other forms of PCD.

Therefore, much remains to be done to further elucidate the true role of mitochondrial fission in apoptosis. Critical questions still need to be answered. In particular, how is physiological fission, which occurs frequently in healthy cells, different from fragmentation of the mitochondrial network occurring during apoptosis? Are pro-apoptotic Bcl-2 proteins implicated in activating mitochondrial fragmentation during apoptosis and what is the mechanism involved? How critical is the requirement for mitochondrial fission in the release of pro-apoptogenic proteins from the mitochondria and in cell death progression? How much of the experimental evidence supporting a role of mitochondrial fission in apoptosis is due to mitochondrial dysfunction resulting from the use of tools that inhibit fission or activate fusion?

In view of the remarkable efforts that have been devoted to elucidate the role of mitochondrial fragmentation in apoptosis, it seems likely that these pending questions will soon be answered. Luckily however, the field of mitochondrial dynamics has a life after cell death and many fundamental questions concerning the role of mitochondrial morphological changes in cellular homeostasis and mitochondrial function still remain unanswered. Addressing these questions is likely to widen our understanding of the mitochondria in ways that go beyond apoptosis.

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