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Abstract

Mitochondria play an important role in the progression of apoptosis through the release of pro-apoptotic factors, such as cytochrome *c,* from the mitochondrial intermembrane space. During this process, mitochondrial networks are dramatically reorganised from long filamentous interconnected tubules into small punctate spheres. Whether remodelling of mitochondrial networks is necessary for apoptosis-associated cytochrome *c* release, or merely an accompanying process, has been a subject of debate. Here we discuss evidence for and against the role of mitochondrial fragmentation in the progression of apoptosis and highlight recent advances which indicate that mitochondrial fission is not a critical requirement for apoptosis-associated cytochrome *c* release. We also discuss an emerging role for Bcl-2 family members as regulators of mitochondrial fission and fusion dynamics, independent of the role of this family in the regulation of apoptosis.

Keywords: Apoptosis, Bcl-2 family, cell death, cytochrome *c*, mitochondrial fission, mitochondrial fusion.

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1. Introduction

Apoptosis is a mode of programmed cell death that is crucial for mammalian development and also plays an essential role in cellular homeostasis and defence against infection. Programmed cell death facilitates the removal of damaged, infected or superfluous cells in a controlled manner that causes minimum disruption to neighbouring cells and minimizes inflammation. Because apoptosis is important for maintaining cell numbers at equilibrium in the adult, deregulation of this process may contribute to the development of neurodegenerative disorders and cancer (Fadeel and Orrenius, 2005).

At a molecular level, apoptosis is regulated by two protein families: the Bcl-2 family which is involved in the initiation phase of apoptosis, and the caspase family of proteases that are responsible for the execution phase (Adams and Cory, 2007; Taylor et al., 2008). As we shall discuss below, mitochondrial cytochrome *c* plays an important role in the propagation of many pro-apoptotic signals through acting as a co-factor for a caspase-activating complex in the cytoplasm, called the apoptosome. As a result, release of cytochrome c from the mitochondrial intermembrane space (IMS) represents an important checkpoint in apoptosis. It is at this checkpoint that the Bcl-2 family exert their regulatory influence on this process.

2. Routes to apoptosis-associated caspase activation

Three main pathways to caspase activation during apoptosis have been well characterized. These are: the extrinsic, intrinsic, and the granzyme B pathways,

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but all pathways ultimately result in the activation of executioner caspases -3 and -7, which promote rapid cell death through proteolysis of key substrates.

The extrinsic pathway is activated most commonly within the immune system and involves binding of TNF family ligands (such as TNF, TRAIL and Fas) to their respective cell surface death receptors, which promotes activation of initiator caspase-8, and subsequent activation of the executioner caspases downstream (Creagh et al., 2003; Taylor et al., 2008). The intrinsic pathway, which is often activated in response to cell stress or damage (such as exposure to cytotoxic drugs, radiation, or elevated temperature), is regulated by the interaction of Bcl-2 family members at mitochondria. Activation of pro-apoptotic Bcl-2 family members during intrinsic cell death leads to the formation of pores in mitochondrial outer membranes, followed by release of cytochrome *c* and other pro-apoptotic factors from the mitochondrial intermembrane space into the cytosol (Youle and Strasser, 2008). Following release, cytochrome c binds APAF-1 triggering a conformational change that permits recruitment of caspase-9 into a complex termed the apoptosome (Hill et al., 2004; Li et al., 1997; Logue and Martin, 2008). Allosteric activation of caspase-9 within the apoptosome then leads to proteolysis and activation of the executioner caspases-3 and –7 downstream (Slee et al., 1999). These, in turn, cleave hundreds of substrates, a small subset of which is responsible for the morphological alterations to the cell architecture that is characteristic of apoptosis (Fischer et al., 2003; Luthi and Martin, 2007). The granzyme B pathway to caspase activation is initiated by cytotoxic T lymphocytes (CTLs) or natural killer (NK) cells to eliminate virally

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infected or cancerous target cells. CTLs and NK cells deliver granzyme B into target cells via a perforin-mediated mechanism and cleavage of caspase-3 by granzyme B within the target cell promotes apoptosis (Cullen and Martin, 2008). In addition, both caspase-8 and granzyme B also cleave the Bcl-2 family member Bid resulting in activation of the intrinsic pathway to apoptosis (Li et al., 1998; Sutton et al., 2000).

3. The Bcl-2 family regulate mitochondrial permeabilization

The Bcl-2 family is comprised of three subgroups; the anti-apoptotic Bcl-2 family members (Bcl-2, Bcl-xL, Mcl-1, A1, Bcl-b, Bcl-w), the pro-apoptotic BH3-only proteins (Bid, Bad, Bim, Bmf, Bik, Noxa, Puma, Hrk), and the pro-apoptotic Bax/Bak sub-family (Figure 1). BH3-only proteins couple cell death signals to mitochondria where the interplay of various members of the Bcl-2 family determines the fate of the cell (Labi et al., 2006). Healthy cells contain high relative amounts of free anti-apoptotic Bcl-2 family members that bind and sequester pro-apoptotic Bax and Bak. In response to cellular stress, BH3-only proteins are activated, either through transcriptional upregulation or post-translational modification, and bind to anti-apoptotic family members, thereby freeing Bax and Bak to promote cytochrome *c* release (Kuwana et al., 2003). Certain BH3-only proteins, namely Bid and Bim, can also interact directly with Bax and Bak, triggering transformation from an inactive to an active conformer (Kim et al., 2009; Kuwana et al., 2005; Merino et al., 2009). Once activated, Bax and Bak then oligomerise into homo- or hetero-oligomers and form pores in the

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mitochondrial outer membrane, which facilitates the release of cytochrome *c* and downstream caspase activation (Chipuk and Green, 2008; Leber and Andrews, 2007; Figure 1).

4. Mitochondrial cytochrome c release during apoptosis

Release of cytochrome *c* from mitochondria is a defining event during apoptosis, as cells will die due to mitochondrial dysfunction even if downstream caspases are inhibited (Colell et al., 2007; Ekert et al., 2004). Cytochrome *c* is confined to the mitochondrial intermembrane space in healthy cells where a large percentage is sequestered into pockets formed by folds in the inner mitochondrial membrane, called cristae (Ow et al., 2008). During apoptosis, cytochrome *c* is released from mitochondria in a rapid, coordinated manner with complete release from all mitochondria occurring within minutes (Goldstein et al., 2000). Pro-apoptotic Bax and/or Bak are both sufficient and essential for this crucial step in the apoptotic process as incubation of Bax and Bak with isolated mitochondria triggers cytochrome *c* release, while simultaneous knockout of both these proteins renders cells resistant to a wide range of pro-apoptotic stimuli (Eskes et al., 1998; Wei et al., 2001). Exactly how these proteins orchestrate mitochondrial outer membrane permeabilisation (MOMP) is still unclear. Current evidence suggests that exposure of the BH3 domain of Bax and Bak during activation presents it for interaction with the hydrophobic groove formed by the BH1 domain in an adjoining molecule, which promotes dimerisation (Dewson et al., 2008; George et al., 2007). Dimers further oligomerise facilitating the formation of a

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pore within mitochondrial outer membranes by a mechanism that is still undefined but may depend on the central region of the protein (α-helices 5 and 6) that resembles bacterial pore-forming proteins (Dewson et al., 2009; Korsmeyer et al., 2000). In addition, Bax and Bak activation by Bid may also trigger changes in mitochondrial cristae structure facilitating the release of cytochrome *c* from inner membrane cristae into the IMS (Yamaguchi et al., 2008).

Concurrent with cytochrome *c* release, the mitochondrial network undergoes dramatic fragmentation from an extended filamentous network into small punctate organelles. This has led to the suggestion that mitochondrial fragmentation promotes Bax/Bak-dependent cytochrome *c* release. Here we will discuss how mitochondria are remodelled during apoptosis and what role this may have on Bax/Bak dependent MOMP.

5. Mitochondrial dynamics

Mitochondria are highly dynamic organelles that are constantly elongating and dividing to form a network that spans the entire area of the cell (Detmer and Chan, 2007). The dynamic nature of mitochondrial networks is due to two opposing processes, mitochondrial fission and fusion, that operate concurrently (Cerveny et al., 2007; Chan, 2006). Mitochondrial fission and fusion are crucial for maintaining mitochondrial function and are thought to be important for rapid repair of damaged mitochondria and for intermixing of DNA and proteins between mitochondria (Chan, 2006). Thus, proteins that play an important role in these processes are important for the maintenance of healthy mitochondria. In addition

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to mitochondrial remodelling via fission and fusion, mitochondria are also transported within the cell along cytoskeletal tracks (Frederick and Shaw, 2007). Studies using neuronal cells have demonstrated that mitochondria translocate towards axonal areas with high metabolic demands, such as synaptic sites. Here, movement of mitochondria in the anterograde direction (towards the axon terminals) occurs along microtubule tracks and depends on kinesin motors, while movement in the retrograde direction (towards the cell body) occurs along actin tracks and utilises dynein motors (Hollenbeck and Saxon, 2005). These movements are aided by a number of signalling and adaptor molecules such as Miro and Milton.

5.1 Mitochondrial fusion

Mitochondrial fusion involves the tethering of two adjacent mitochondria followed by merging, or fusion, of the inner and outer mitochondrial membranes (Figure 2). This facilitates the exchange of materials between these organelles and aids repair of defective mitochondria. Efficient mitochondrial fusion is important as cells defective for fusion display reduced cell growth, decreased mitochondrial membrane potential and defective respiration (Chen et al., 2005). Studies in *D. melanogaster* and yeast have identified Fzo1 and Mgm1 as the major players in mitochondrial fusion (Griffin et al., 2006; Okamoto and Shaw, 2005). The mammalian homologues of Fzo1 are Mfn1 and Mfn2, two large GTPases that are localised on the mitochondrial outer membrane (Eura et al., 2003; Santel et al., 2003). C-terminal coiled-coil domains facilitate homo- or heterodimeric

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interactions between these two proteins on adjacent mitochondria, which promotes tethering and GTPase-dependent fusion of mitochondrial outer membranes (Koshiba et al., 2004; Santel et al., 2001; Santel et al., 2003). Studies utilising knockout mice have demonstrated the importance of Mfn1 and Mfn2 for mitochondrial fusion as loss of both proteins leads to gross mitochondrial fragmentation due to impaired fusion (Chen et al., 2003). However, Mfn1 and Mfn2 are partially redundant as reconstitution of either protein reversed the fragmented phenotype to some extent. While mitofusins are important for fusion of the outer mitochondrial membrane, Opa1, the mammalian homologue of Mgm1, is crucial for the fusion of inner mitochondrial membranes. Opa1 is a dynamin-related protein that is situated on the mitochondrial inner membrane and ablation of this protein also inhibits mitochondrial fusion (Olichon et al., 2002). Evidence also suggests that Opa1 has an important role to play in maintaining mitochondrial cristae structure as loss of this protein results in disorganisation of cristae and widening of cristae junctions (Arnoult et al., 2005a; Frezza et al., 2006; Olichon et al., 2003; Yamaguchi et al., 2008).

5.2 Mitochondrial fission

Mitochondrial fission depends largely on the dynamin related protein Drp1, which is localised predominantly in the cytosol and must be recruited to mitochondria for fission to occur (Smirnova et al., 1998; Smirnova et al., 2001; Figure 2). Translocation of Drp1 in yeast is facilitated by it's receptor, Fis1, which is tethered to the mitochondrial outer membrane. Human Fis1 may also be

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responsible for recruiting Drp1 to mitochondria in mammals (James et al., 2003). However, translocation of Drp1 still occurs in Fis1 ablated cells and direct interaction between endogenous human Drp1 and Fis1 has yet to be shown indicating that other receptors may also exist (Lee et al., 2004). Current evidence suggests that Drp1 promotes fission by tethering to mitochondria at specific positions known as constriction sites. Drp1 then forms multimeric spirals around mitochondria further constricting mitochondrial tubules leading to mitochondrial fission (Smirnova et al., 2001). Ablation of Drp1 with siRNA, or overexpression of a dominant negative form of Drp1, Drp1 K38A, have demonstrated the crucial role of this protein in mediating mitochondrial fission as these cells contain highly elongated, fused mitochondria (Lee et al., 2004; Smirnova et al., 2001). Two additional fission proteins, Mdv1 and Caf4, which are involved in the recruitment of Drp1 from the cytosol to mitochondria were identified in yeast, although mammalian homologues of these proteins have not been identified thus far. However, other proteins have been implicated in mitochondrial fission in humans such as MPT18, Endophilin B1 and GDAP1 and these may functionally replace yeast Mdv1 and Caf4 (Karbowski et al., 2004; Niemann et al., 2005; Tondera et al., 2004).

5.3 Mitochondrial fission/fusion dynamics and disease

Efficient mitochondrial function is crucial for the maintenance of healthy cells and thus, disruption of mitochondrial fission and fusion has been linked to the development and progression of some diseases. Neurons appear to be

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particularly dependent on mitochondria for ATP production and calcium signalling and these cells are more sensitive to perturbations of mitochondrial function. Mutation of Opa1 has been identified as a major cause of Dominant Optic Atrophy which affects the optic nerves (Delettre et al., 2000), while mutation of Mfn2 leads to Charcot-Marie-Tooth neuropathy type 2A, a peripheral neuropathy that affects motor and sensory neurons (Verhoeven et al., 2006; Zuchner et al., 2004). In addition, increased mitochondrial fission and decreased fusion has been implicated in the progression of Huntington's disease and Alzheimer's disease (Chen and Chan, 2009; Su et al., 2010). Furthermore, recent studies have revealed that Parkin and Pink1, proteins involved in the development of Parkinson's disease, play a crucial role in the removal of defective mitochondria through mitophagy (Narendra et al., 2009; Geisler et al., 2010). Thus, because defects in mitochondrial dynamics are known to promote neurodegenerative diseases, understanding the function of mitochondrial fission and fusion regulators will aid elucidation of their role in these diseases.

6. Mitochondrial fission during apoptosis

As described previously, the release of cytochrome *c* from mitochondria is a crucial step in apoptosis. Within a similar time frame, mitochondria fragment from filamentous tubules into numerous small punctate particles (Frank et al., 2001; Gao et al., 2001; Jahani-Asl et al., 2007; Lee et al., 2004; Sheridan et al., 2008; Figure 2). These fragmented mitochondria often collapse from an extended network covering the majority of the cell, into a clustered perinuclear

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pattern (De Vos et al., 1998; Sheridan et al., 2008; Figure 3). Fragmented mitochondria also display decreased and non-directed motility when compared to the behaviour of mitochondria within healthy cells (Sheridan et al., 2008). Drp1 appears to be responsible for this fragmented phenotype as studies have demonstrated that ablation of Drp1 reduces mitochondrial fragmentation during apoptosis (Estaquier et al., 2007; Frank et al., 2001; Karbowski et al., 2002; Sugioka et al., 2004), while overexpression of dominant negative Drp1 also prevents apoptosis-induced mitochondrial fragmentation (Arnoult et al., 2005b; Frank et al., 2001). In addition, increased recruitment of Drp1 to mitochondrial sission sites during apoptosis has been demonstrated (Cassidy-Stone et al., 2008; Frank et al., 2001).

So what triggers Drp1 translocation to mitochondria and mitochondrial fission during death? An interesting study by Strack and colleagues demonstrated that Drp1 is constitutively phosphorylated by cyclic AMP-dependent protein kinase and that this modification restricts the activity of Drp-1 (Cribbs et al., 2007). However, following a death stimulus such as staurosporine, the phosphatase calcineurin dephosphorylates Drp-1, triggering its translocation from the cytosol to mitochondria, thus increasing fission (Cereghetti et al., 2008; Cribbs et al., 2007). In addition, overexpression of the BH3-only protein Bik induces Drp1-dependent mitochondrial fission by a pathway dependent on calcium signalling (Germain et al., 2005). Thus in response to some apoptotic stimuli, increased intracellular Ca^{2+} levels during the early stages of apoptosis may encourage calcineurin-dependent Drp1 translocation. Furthermore,

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intracellular Ca^{2+} increases have also been linked to reduced mitochondrial movement along cytoskeletal tracks and may contribute to the impaired motility of fragmented mitochondria during cell death (Sheridan et al., 2008; Wang et al., 2009). Increased fission by Drp1 has also been linked to the release of DDP from mitochondria during MOMP (Arnoult et al., 2005b). This facilitates binding of DDP to Drp1 and augmented recruitment of Drp1 to mitochondrial sission sites (Arnoult et al., 2005b). Other reports have indicated that Bax and Bak may be involved in Drp1 dependent mitochondrial fission. Co-localisation of Bax with Drp1 and Mfn2 at mitochondrial fission sites has been demonstrated, however it is unclear whether this promotes Drp1-mediated fission (Karbowski et al., 2002). Another study has indicated that Drp1 accumulates on mitochondria during apoptosis due to Bax/Bak-dependent sumolylation of Drp1, which results in stable membrane association of Drp1 with mitochondria and thus increased fission (Wasiak et al., 2007; Zunino et al., 2007). There is also evidence to suggest that mitochondrial fusion is blocked upon activation of apoptosis indicating that the fragmented phenotype may occur due to a combination of increased fission and decreased fusion (Karbowski et al., 2004).

7. The role of mitochondrial fission in apoptosis

While fragmentation of mitochondria during apoptosis is widely agreed upon, whether this event drives mitochondrial outer membrane permeabilisation has been disputed (Table 1). Initial observations by Youle and colleagues indicated that overexpression of a dominant negative form of Drp1 had a protective effect

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against cytochrome *c* release and apoptosis in some contexts (Frank et al., 2001). Other groups reported similar findings, while ablation of Drp1 was also shown to reduce cytochrome *c* release (Breckenridge et al., 2003; Brooks et al., 2007; Germain et al., 2005; Lee et al., 2004; Neuspiel et al., 2005). These results suggested that inhibition of Drp1-mediated fission prevents progression of apoptosis. Conversely, two additional studies demonstrated that while ablation of Drp1 partially prevented cytochrome *c* release, death was unaffected (Estaquier et al., 2007; Parone et al., 2006). Moreover, although cytochrome *c* release was delayed under these conditions, the release of other mitochondrial intermembrane space proteins, such as Smac/Diablo, was unaffected demonstrating that MOMP proceeded without hindrance.

More recently, examination of the ability of Drp1 K38A to inhibit apoptosis in response to a wide range of apoptotic stimuli, at various timepoints, saw little effect on cytochrome *c* release and no decrease in apoptosis (Sheridan et al., 2008). Finally, a chemical inhibitor of Drp1 which reduced staurosporin-mediated apoptosis, also prevented tBid-induced cytochrome *c* release from isolated mitochondria that do not undergo fragmention (Cassidy-Stone et al., 2008). This suggests that the possible pro-apoptotic role of Drp1 may not be related to modulation of mitochondrial fission. Meanwhile, ablation of the fission mediator Fis1 has been shown to reduce apoptosis-mediated cytochrome *c* release, but surprisingly, Bax activation and translocation from the cytosol to mitochondria did not occur under these conditions. This suggests a role for Fis1 in promoting apoptosis upstream of mitochondrial fission, indicating that Fis1 may somehow

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be involved in Bax recruitment to mitochondria (Lee et al., 2001).

The role of mitochondrial fragmentation in the progression of apoptosis has also been investigated by inhibiting fission through enforced fusion. Overexpression of Mfn2 has been reported to inhibit cytochrome *c* release but the effect on apoptosis was not addressed (Neuspiel et al., 2005). In other studies overexpression of Mfn2 and the rat homologue Fzo1 reduced cytochrome *c* release and apoptosis (Jahani-Asl et al., 2007; Sugioka et al., 2004) while in a fourth study, overexpression of Mfn1, Mfn2 or Opa1 had no effect on cytochrome *c* release or apoptosis (Sheridan et al., 2008). Importantly, the authors of the latter study demonstrated that cells displaying fused phenotypes still lost cytochrome *c* in response to pro-apoptotic stimuli, indicating that Bax/Bak-dependent pore formation can still occur in fused mitochondria. Similarly, overexpression of *C. elegans* CED-9 (a Bcl-2 homologue) in mammalian cells caused mitochondrial fusion but provided no protection against Bax-induced cytochrome c release and apoptosis (Delivani et al., 2006). Additionally, cells with reduced levels of Mfn2 showed no augmentation in cytochrome *c* release, indicating that inhibition of mitochondrial fusion is not a pre-requisite to MOMP (Arnoult et al., 2005a).

Overexpression of the mitochondrial fission protein Fis-1 induces apoptosis in some cell types, which suggests that mitochondrial fission may be a driver of apoptosis (Alirol et al., 2006; James et al., 2003; Yu et al., 2005). However, Fis-1-mediated cell death is inhibited by Bcl-xL overexpression and is Bax/Bak-dependent, demonstrating that the cells die due to extensive

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mitochondrial dysfunction rather than fission-induced mitochondrial permeabilisation (Alirol et al., 2006; Yu et al., 2005). Similar results have been demonstrated with Opa1 ablation, which results in mitochondrial fragmentation and apoptosis that is inhibited by Bcl-2, again indicating that death is triggered by stress due to Opa1 loss rather than fragmentation-induced cytochrome *c* release (Lee et al., 2004; Olichon et al., 2003). Furthermore, many groups have observed dramatically fragmented mitochondria in healthy cells, indicating that mitochondrial fission alone does not necessarily result in cell death (Chen et al., 2004; Delivani et al., 2006; De Vos et al., 2005; Karbowski et al., 2006; Norris et al., 2008; Sheridan et al., 2008; Szabadkai et al., 2004; Taguchi et al., 2007).

8. Bax/Bak-dependent mitochondrial fission can be uncoupled from apoptosis

More recently, the ability of Bax and Bak to promote mitochondrial fragmentation has been separated from their role in MOMP by co-expression with anti-apoptotic Bcl-2 family members (Sheridan et al., 2008). Overexpression of Bax and Bak leads to mitochondrial fission, cytochrome c release and apoptosis. However when Bax or Bak were co-expressed with anti-apoptotic Bcl-2 family members such as Bcl-xL and Mcl-1, mitochondrial fragmentation still occurred but cytochrome *c* release and apoptosis were prevented (Sheridan et al., 2008; Figure 2). This indicates that while Bax and Bak promote apoptosis through MOMP they may also disrupt the balance of mitochondrial fission and fusion, thus resulting in mitochondrial fragmentation. These observations argue that

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mitochondrial fission merely accompanies cytochrome *c* release, rather than orchestrating events that lead to release of pro-apoptotic molecules from the mitochondrial inner membrane space.

Emerging evidence indicates that in addition to promoting mitochondrial inner membrane fusion, Opa1 also regulates mitochondrial cristae structure. As cytochrome *c* is found pre-dominantly within cristae, this places Opa1 in a prime position to regulate its release. Ablation of Opa1 in a number of studies has resulted in dramatic disorganisation of mitochondrial cristae, accompanied by accelerated cytochrome *c* release in response to tBid, indicating that there may be an increased availability of cytochrome *c* in the intermembrane space in these cells (Arnoult et al., 2005a; Olichon et al., 2003). This observation has been further validated by the identification of an Opa1 mutant that blocks cytochrome *c* release following an apoptotic stimulus by preventing cristae junction opening (Yamaguchi et al., 2008). Opa1 is thought to form oligomers in the inner mitochondrial membrane consisting of full length membrane bound Opa1 and short soluble forms that have been cleaved in a Parl-dependent manner (Cipolat et al., 2006; Frezza et al., 2006). During apoptosis, activation of Bax and Bak is accompanied by disruption of Opa1 oligomers and release of Opa1 from mitochondria (Arnoult et al., 2005a; Yamaguchi et al., 2008). This loss of Opa1 may play a role in cristae junction opening, thus releasing sequestered cytochrome *c* and may also cause mitochondrial fission due to impairment of fusion. However, although Opa1 loss may enhance or strengthen an apoptotic signal by providing additional cytochrome *c*, it is likely that the small percentage

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of cytochrome *c* that is available in the intermembrane space is sufficient for apoptosome assembly and cell death. Thus Opa1 may affect the kinetics of apoptosis but is unlikely to prevent death itself.

9. Mitochondrial fission during apoptosis in lower organisms

A number of studies have addressed the issue of whether mitochondrial fission is important for programmed cell death in lower organisms such as *S. cerevisae*, *C. elegans* and *D. melanogaster* (Breckenridge et al., 2008; Fannjiang et al., 2004; Goyal et al., 2007; Jagasia et al., 2005). While ablation of Dnm1 in yeast and Drp1 in flies increased survival following death stimuli, overexpression of dominant negative Drp1 reduced developmental cell death in nematodes. However it is hard to reconcile these data with the lack of evidence for a requirement for cytochrome *c* or other mitochondrial factors for cell death in these organisms (Dorstyn et al., 2004). Indeed a more recent study by Xue and colleagues comprehensively showed that loss of function mutations in fission and fusion genes in *C. elegans* had no effect on apoptotic cell death (Breckenridge et al., 2008). Instead they found that Drp1 and Fis1 promote the elimination of mitochondria in apoptotic cells indicating that these proteins play a role in the execution phase of apoptosis rather than the initiating phase. So why is mitochondrial fission detected in *C. elegans* upstream of CED-3 activation? The answer may lie with the Bcl-2 family of proteins. Fragmentation of mitochondria in nematodes is induced by the BH3-only protein EGL-1 and inhibited by CED-9 gain of function mutants. It is possible that binding of EGL-1 to CED-9 disrupts

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an alternative function of CED-9 in promoting mitochondrial fusion, as discussed in greater detail below.

10. Mitochondrial fission as a consequence rather than a cause of MOMP

Although mitochondrial fragmentation accompanies cytochrome *c* release, the separation of these two events during Bax-induced apoptosis demonstrates that they are not inter-dependent steps. Because cytochrome *c* release can occur in cells displaying fused or elongated mitochondria, fission does not appear to be required for Bax/Bak pore formation. Moreover, timelapse microscopy in cells treated with pro-apoptotic drugs demonstrated that cytochrome *c* release precedes mitochondrial fragmentation by at least ten minutes suggesting that fission might occur as a consequence of MOMP (Arnoult et al., 2005a; Gao et al., 2001). Thus, apoptosis-associated mitochondrial fission may passively promote mitochondrial network disassembly, rather than playing an important regulatory role in apoptosis. There is also evidence that caspases target mitochondria during apoptosis, resulting in loss of mitochondrial inner membrane potential as a result of proteolysis of proteins important for mitochondrial respiratory function (Dinsdale et al., 1999; Loucks et al., 2009; Ricci et al., 2004; Sun et al., 2007). Therefore, mitochondrial fragmentation may be an early event in the demolition of mitochondria that is concluded by caspases in the later stages of apoptosis.

11. Modulation of mitochondrial dynamics by Bcl-2 family members

While the function of Bcl-2 family members in cell death regulation is well

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understood, a new housekeeping role for Bcl-2-related proteins in modulating mitochondrial dynamics is emerging. One of the the first indications of this secondary role for Bcl-2 proteins came from a study expressing the *C. elegans* anti-apoptotic Bcl-2 family member, CED-9, in mammalian cells. Strikingly, while overexpression of CED-9 was unable to prevent Bax-induced cytochrome *c* release and apoptosis, it did cause dramatic remodelling of the mitochondrial network from long filamentous tubules distributed throughout the cell to fused mitochondria, clustered around the nucleus (Delivani et al., 2006). One of the mammalian homologues of CED-9, Bcl-xL, similarly induced mitochondrial fusion and both of these proteins were shown to interact with Mfn2, suggesting that they may enhance Mfn2-mediated fusion. Interestingly, co-expression of EGL-1 with CED-9 reversed the fused phenotype, giving rise to fragmented mitochondria (but not cell death or cytochrome c release), suggesting that pro-apoptotic members of the Bcl-2 family may cause mitochondrial fission during apoptosis via inhibiting anti-apoptotic Bcl-2 protein-mediated fusion.

Additional studies in neurons have since described a role for Bcl-2 family members in regulation of mitochondrial dynamics. While Bcl-w deficiency in the brain produces no cell death defects, mitochondria in purkinje cells are elongated and these cells have abnormal synapses and dendrites, possibly due to impaired mitochondrial fission (Liu et al., 2008). Bcl-xL has also been implicated in regulating mitochondrial morphology in neurons. Expression of Bcl-xL in neurons results in increased mitochondrial fission and fusion and also increased mitochondrial biomass, while conditional knockout of Bcl-xL in cortical neurons

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resulted in a fragmented phenotype (Berman et al., 2008). Expression of Bcl-xL has also been linked with elevated numbers of neuronal synapses due to enhanced mitochondrial localisation at synaptic sites (Li et al., 2008). These effects are thought to be mediated by mitochondrial fission in a Drp1-dependent manner. In addition to the anti-apoptotic members of the Bcl-2 family, pro-apoptotic Bax and Bak have also been linked to regulation of mitochondrial morphology. Double knockout of Bax and Bak in mouse embryonic fibroblasts gives rise to fragmented mitochondria, indicating that these proteins have a role in mitochondrial fusion (Karbowski et al., 2006). However, overexpression of Bax and Bak similarly induces mitochondrial fission (Sheridan et al., 2008). Thus, changes in the ratios of Bax and Bak versus anti-apoptotic Bcl-2 family members or other fission and fusion mediators may influence mitochondrial morphology.

So how do Bcl-2 family members regulate mitochondrial morphology? Because none of the Bcl-2 family proteins contain a GTPase domain, utilised by the well-defined fission and fusion regulators to modulate mitochondrial fission/fusion, it is unlikely that Bcl-2 family members directly affect mitochondrial morphology. Instead these proteins may act more distally as adaptors or facilitators of mitochondrial fission and fusion. The localisation of many Bcl-2 family members such as Bcl-2, Bcl-xL and Bak on mitochondrial outer membranes places them in an ideal location to modulate the activity and interactions of other fusion and fission mediators. In line with this theory, a number of interactions between Bcl-2-related proteins and fission and fusion proteins have been documented. Bcl-xL binds to both Mfn2 and Drp1 in different

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cell types, which may explain the fission and fusion phenotypes seen with overexpression of Bcl-xL (Berman et al., 2008; Delivani et al., 2006; Li et al., 2008; Sheridan et al., 2008). Bak has been shown to interact with Mfn1 and Mfn2, while Bax promotes the activity of Mfn2 (Brooks et al., 2007, Karbowski et al., 2006). In addition, Bcl-2 family members may interact with other cellular proteins that modulate mitochondrial morphology and distribution within the cell. Unfortunately, examination of the role of these proteins, particularly Bax and Bak, in modulating mitochondrial morphology is hampered by their apoptosis-inducing properties. Hence, separating the apoptotic functions of the pro- and anti-apoptotic Bcl-2 family members from their ability to modulate mitochondrial morphology will be the key to dissecting this secondary role.

While the mechanism employed by Bcl-2 family members to regulate mitochondrial fission and fusion dynamics is still ambiguous, this may be an important, conserved function of these proteins. *C. elegans* CED-9 also modulates mitochondrial dynamics indicating that this may be an ancient function of the Bcl-2 family (Delivani et al., 2006; Rolland et al., 2009; Tan et al., 2008). Although loss-of-function mutants in CED-9 revealed no dramatic alterations in mitochondrial morphology, overexpression of CED-9 in *C. elegans* muscle cells resulted in highly interconnected mitochondria, while loss-of-function mutants enhanced Drp-1 mediated fragmentation (Breckenridge et al., 2009; Tan et al., 2008). This argues that while CED-9 does not directly mediate mitochondrial fission or fusion, it probably promotes or inhibits the activity of other fission and fusion regulators. A role for CED-9 in regulating mitochondrial dynamics

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provides a possible explanation for the localisation of CED-9 on mitochondrial outer membranes in *C. elegans*, where no role for cytochrome c or mitochondria in apoptotic cell death is known. This may also be an important function for *Drosophila* Bcl-2 family members that play a limited role in apoptosis in the fly (Galindo et al., 2009; Sevrioukov et al., 2007).

12. Conclusions

In conclusion, although it is clear that mitochondrial fragmentation is a widespread phenomenon during apoptosis, it is unlikely that this event is crucial to the progression of programmed cell death. In contrast to anti-apoptotic Bcl-2 family members, modulation of fission and fusion proteins cannot provide cells with robust protection against a death stimulus. The defining event resulting in cytochrome c release from mitochondria is the formation of Bax/Bak pores and, to date, there is no evidence to suggest that fission or fusion of mitochondria impacts on pore formation. Therefore, apoptosis is likely to proceed regardless of the mitochondrial phenotype. However, as mitochondrial fragmentation is a conserved event seen during death in many different organisms, it may be important for the dismantling and removal of these organelles in dying cells. Anti-apoptotic Bcl-2 family members have been shown to perturb mitochondria in the absence of any cell death phenotype hinting that these proteins may have a role in mitochondrial remodelling within healthy cells that is separate to their role in protecting against apoptosis (Autret and Martin, 2009; Berman et al., 2008; Delivani et al., 2006; Liu et al., 2008; Sheridan et al., 2008). Thus, increased

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interactions with BH3-only proteins during apoptosis may disrupt their ability to regulate mitochondrial fusion culminating in the fragmented phenotype so often seen in death. In the future, examination of the interplay between Bcl-2-related proteins and members of the fission and fusion machinery may enhance our understanding of the role of the Bcl-2 family in mitochondrial fission and fusion. Thus, dissecting the part played by Bcl-2 family members in the regulation of mitochondrial morphology in healthy cells may be the key to understanding what triggers mitochondrial fission during apoptosis.

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References

Adams, J.M., and Cory, S., 2007. Bcl-2-regulated apoptosis: mechanism and therapeutic potential. Curr. Opin. Immunol. 19, 488-496.

Alirol, E., James, D., Huber, D., Marchetto, A., Vergani, L., Martinou, J.C., and Scorrano, L., 2006. The mitochondrial fission protein hFis1 requires the endoplasmic reticulum gateway to induce apoptosis. Mol. Biol. Cell 17, 4593-4605.

Arnoult, D., Grodet, A., Lee, Y.J., Estaquier, J., and Blackstone, C., 2005a. Release of OPA1 during apoptosis participates in the rapid and complete release of cytochrome c and subsequent mitochondrial fragmentation. J. Biol. Chem. 280, 35742-35750.

Arnoult, D., Rismanchi, N., Grodet, A., Roberts, R.G., Seeburg, D.P., Estaquier, J., Sheng, M., and Blackstone, C., 2005b. Bax/Bak-dependent release of DDP/TIMM8a promotes Drp1-mediated mitochondrial fission and mitoptosis during programmed cell death. Current Biology 15, 2112-2118.

Autret, A., and Martin, S.J., 2009. Emerging role for members of the Bcl-2 family in mitochondrial morphogenesis. Molecular Cell 36, 355-63.

Berman, S.B., Chen, Y.B., Qi, B., McCaffery, J.M., Rucker, E.B., 3rd, Goebbels, S., Nave, K.A., Arnold, B.A., Jonas, E.A., Pineda, F.J., and Hardwick, J.M., 2009. Bcl-x L increases mitochondrial fission, fusion, and biomass in neurons. J. Cell. Biol. 184, 707-719.

Breckenridge, D.G., Kang, B.H., Kokel, D., Mitani, S., Staehelin, L.A., and Xue, D., 2008. Caenorhabditis elegans drp-1 and fis-2 regulate distinct cell-death execution pathways downstream of ced-3 and independent of ced-9. Molecular Cell 31, 586-597.

Breckenridge, D.G., Kang, B.H., and Xue, D., 2009. Bcl-2 proteins EGL-1 and CED-9 do not regulate mitochondrial fission or fusion in Caenorhabditis elegans. Current Biology 19, 768-773.

Breckenridge, D.G., Stojanovic, M., Marcellus, R.C., and Shore, G.C., 2003. Caspase cleavage product of BAP31 induces mitochondrial fission through endoplasmic reticulum calcium signals, enhancing cytochrome c release to the

ACCEPTED MANUSCRIPT

cytosol. J. Cell Biol. 160, 1115-1127.

Brooks, C., Wei, Q., Feng, L., Dong, G., Tao, Y., Mei, L., Xie, Z.J., and Dong, Z., 2007. Bak regulates mitochondrial morphology and pathology during apoptosis by interacting with mitofusins. Proc. Natl. Acad. Sci. U S A 104, 11649-11654.

Cassidy-Stone, A., Chipuk, J.E., Ingerman, E., Song, C., Yoo, C., Kuwana, T., Kurth, M.J., Shaw, J.T., Hinshaw, J.E., Green, D.R., and Nunnari, J., 2008. Chemical inhibition of the mitochondrial division dynamin reveals its role in Bax/Bak-dependent mitochondrial outer membrane permeabilization. Developmental Cell 14, 193-204.

Cereghetti, G.M., Stangherlin, A., Martins de Brito, O., Chang, C.R., Blackstone, C., Bernardi, P., and Scorrano, L., 2008. Dephosphorylation by calcineurin regulates translocation of Drp1 to mitochondria. Proc. Natl. Acad. Sci. U S A 105, 15803-15808.

Cerveny, K.L., Tamura, Y., Zhang, Z., Jensen, R.E., and Sesaki, H., 2007. Regulation of mitochondrial fusion and division. Trends Cell Biol. 17, 563-569.

Chan, D.C., 2006. Mitochondrial fusion and fission in mammals. Annu. Rev. Cell Dev. Biol. 22, 79-99.

Chen, H. and Chan, D.C., 2009. Mitochondrial dynamics--fusion, fission, movement, and mitophagy--in neurodegenerative diseases. Hum Mol Genet. 18, 169-76.

Chen, H., Chomyn, A., and Chan, D.C., 2005. Disruption of fusion results in mitochondrial heterogeneity and dysfunction. J. Biol. Chem. 280, 26185-26192.

Chen, H., Detmer, S.A., Ewald, A.J., Griffin, E.E., Fraser, S.E., and Chan, D.C., 2003. Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. J. Cell Biol. 160, 189-200.

Chipuk, J.E., and Green, D.R., 2008. How do BCL-2 proteins induce mitochondrial outer membrane permeabilization? Trends Cell Biol. 18, 157-164.

Cipolat, S., Rudka, T., Hartmann, D., Costa, V., Serneels, L., Craessaerts, K., Metzger, K., Frezza, C., Annaert, W., D'Adamio, L., Derks, C., Dejaegere, T.,

ACCEPTED MANUSCRIPT

Pellegrini, L., D'Hooge, R., Scorrano, L., and De Strooper, B., 2006. Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. Cell 126, 163-175.

Colell, A., Ricci, J.E., Tait, S., Milasta, S., Maurer, U., Bouchier-Hayes, L., Fitzgerald, P., Guio-Carrion, A., Waterhouse, N.J., Li, C.W., Mari, B., Barbry, P., Newmeyer, D.D., Beere, H.M., and Green, D.R., 2007. GAPDH and autophagy preserve survival after apoptotic cytochrome c release in the absence of caspase activation. Cell 129, 983-997.

Creagh, E.M., Conroy, H., and Martin, S.J., 2003. Caspase-activation pathways in apoptosis and immunity. Immunol. Rev. 193, 10-21.

Cribbs, J.T., and Strack, S., 2007. Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. EMBO Reports 8, 939-944.

Cullen, S.P., and Martin, S.J., 2008. Mechanisms of granule-dependent killing. Cell Death Differ 15, 251-62.

De Vos, K., Goossens, V., Boone, E., Vercammen, D., Vancompernolle, K., Vandenabeele, P., Haegeman, G., Fiers, W., and Grooten, J., 1998. The 55-kDa tumor necrosis factor receptor induces clustering of mitochondria through its membrane-proximal region. J. Biol. Chem. 273, 9673-9680.

De Vos, K.J., Allan, V.J., Grierson, A.J., and Sheetz, M.P., 2005. Mitochondrial function and actin regulate dynamin-related protein 1-dependent mitochondrial fission. Curr. Biol. 15, 678-683.

Delettre, C., Lenaers, G., Griffoin, J.M., Gigarel, N., Lorenzo, C., Belenguer, P., Pelloquin, L., Grosgeorge, J., Turc-Carel, C., Perret, E., Astarie-Dequeker, C., Lasquellec, L., Arnaud, B., Ducommun, B., Kaplan, J., and Hamel, C.P., 2000. Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. Nature Genetics 26, 207-210.

Delivani, P., Adrain, C., Taylor, R.C., Duriez, P.J., and Martin, S.J., 2006. Role for CED-9 and Egl-1 as regulators of mitochondrial fission and fusion dynamics. Molecular Cell 21, 761-773.

Detmer, S.A., and Chan, D.C., 2007. Functions and dysfunctions of mitochondrial

ACCEPTED MANUSCRIPT

dynamics. Nature Reviews Mol. Cell Biol. 8, 870-879.

Dewson, G., Kratina, T., Czabotar, P., Day, C.L., Adams, J.M., and Kluck, R.M., 2009. Bak activation for apoptosis involves oligomerization of dimers via their alpha6 helices. Molecular Cell 36, 696-703.

Dewson, G., Kratina, T., Sim, H.W., Puthalakath, H., Adams, J.M., Colman, P.M., and Kluck, R.M., 2008. To trigger apoptosis, Bak exposes its BH3 domain and homodimerizes via BH3:groove interactions. Molecular Cell 30, 369-380.

Dinsdale, D., Zhuang, J., and Cohen, G.M., 1999. Redistribution of cytochrome c precedes the caspase-dependent formation of ultracondensed mitochondria, with a reduced inner membrane potential, in apoptotic monocytes. Am. J. Pathol. 155, 607-618.

Dorstyn, L., Mills, K., Lazebnik, Y., and Kumar, S., 2004. The two cytochrome c species, DC3 and DC4, are not required for caspase activation and apoptosis in Drosophila cells. J. Cell Biol. 167, 405-410.

Ekert, P.G., Read, S.H., Silke, J., Marsden, V.S., Kaufmann, H., Hawkins, C.J., Gerl, R., Kumar, S., and Vaux, D.L., 2004. Apaf-1 and caspase-9 accelerate apoptosis, but do not determine whether factor-deprived or drug-treated cells die. J. Cell Biol. 165, 835-842.

Eskes, R., Antonsson, B., Osen-Sand, A., Montessuit, S., Richter, C., Sadoul, R., Mazzei, G., Nichols, A., and Martinou, J.C., 1998. Bax-induced cytochrome C release from mitochondria is independent of the permeability transition pore but highly dependent on Mg2+ ions. J. Cell Biol. 143, 217-224.

Estaquier, J., and Arnoult, D., 2007. Inhibiting Drp1-mediated mitochondrial fission selectively prevents the release of cytochrome c during apoptosis. Cell Death Differ. 14, 1086-1094.

Eura, Y., Ishihara, N., Yokota, S., and Mihara, K., 2003. Two mitofusin proteins, mammalian homologues of FZO, with distinct functions are both required for mitochondrial fusion. J. Biochem. 134, 333-344.

Fadeel, B., and Orrenius, S., 2005. Apoptosis: a basic biological phenomenon with wide-ranging implications in human disease. J. Intern. Med. 258, 479-517.

Fannjiang, Y., Cheng, W.C., Lee, S.J., Qi, B., Pevsner, J., McCaffery, J.M., Hill,

ACCEPTED MANUSCRIPT

R.B., Basanez, G., and Hardwick, J.M., 2004. Mitochondrial fission proteins regulate programmed cell death in yeast. Genes Dev. 18, 2785-2797.

Fischer, U., Janicke, R.U., and Schulze-Osthoff, K., 2003. Many cuts to ruin: a comprehensive update of caspase substrates. Cell Death Differ. 10, 76-100.

Frank, S., Gaume, B., Bergmann-Leitner, E.S., Leitner, W.W., Robert, E.G., Catez, F., Smith, C.L., and Youle, R.J., 2001. The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. Developmental Cell 1, 515-525.

Frederick, R.L., and Shaw, J.M., 2007. Moving mitochondria: establishing distribution of an essential organelle. Traffic 8, 1668-1675.

Frezza, C., Cipolat, S., Martins de Brito, O., Micaroni, M., Beznoussenko, G.V., Rudka, T., Bartoli, D., Polishuck, R.S., Danial, N.N., De Strooper, B., and Scorrano, L., 2006. OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. Cell 126, 177-189.

Galindo, K.A., Lu, W.J., Park, J.H., and Abrams, J.M., 2009. The Bax/Bak ortholog in Drosophila, Debcl, exerts limited control over programmed cell death. Development 136, 275-283.

Gao, W., Pu, Y., Luo, K.Q., and Chang, D.C., 2001. Temporal relationship between cytochrome c release and mitochondrial swelling during UV-induced apoptosis in living HeLa cells. J. Cell Sci. 114, 2855-2862.

Geisler, S., Holmström, K.M., Skujat, D., Fiesel, F.C., Rothfuss, O.C., Kahle, P.J., Springer, W., 2010. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol. 12, 119-31.

George, N.M., Evans, J.J., and Luo, X., 2007. A three-helix homo-oligomerization domain containing BH3 and BH1 is responsible for the apoptotic activity of Bax. Genes Dev. 21, 1937-1948.

Germain, M., Mathai, J.P., McBride, H.M., and Shore, G.C., 2005. Endoplasmic reticulum BIK initiates DRP1-regulated remodelling of mitochondrial cristae during apoptosis. Embo Journal 24, 1546-1556.

Goldstein, J.C., Waterhouse, N.J., Juin, P., Evan, G.I., and Green, D.R., 2000. The coordinate release of cytochrome c during apoptosis is rapid, complete

ACCEPTED MANUSCRIPT

and kinetically invariant. Nature Cell Biology 2, 156-162.

Goyal, G., Fell, B., Sarin, A., Youle, R.J., and Sriram, V., 2007. Role of mitochondrial remodeling in programmed cell death in Drosophila melanogaster. Developmental Cell 12, 807-816.

Griffin, E.E., Detmer, S.A., and Chan, D.C., 2006. Molecular mechanism of mitochondrial membrane fusion. Biochim Biophys Acta 1763, 482-489.

Hill, M.M., Adrain, C., Duriez, P.J., Creagh, E.M., and Martin, S.J., 2004. Analysis of the composition, assembly kinetics and activity of native Apaf-1 apoptosomes. Embo Journal 23, 2134-2145.

Hollenbeck, P.J., and Saxton, W.M., 2005. The axonal transport of mitochondria. J. Cell Sci. 118, 5411-5419.

Jagasia, R., Grote, P., Westermann, B., and Conradt, B., 2005. DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in C. elegans. Nature 433, 754-760.

James, D.I., Parone, P.A., Mattenberger, Y., and Martinou, J.C., 2003. hFis1, a novel component of the mammalian mitochondrial fission machinery. J. Biol. Chem. 278, 36373-36379.

Jahani-Asl, A., Cheung, E.C., Neuspiel, M., MacLaurin, J.G., Fortin, A., Park, D.S., McBride, H.M., and Slack, R.S., 2007. Mitofusin 2 protects cerebellar granule neurons against injury-induced cell death. J. Biol. Chem. 282, 23788-23798.

Karbowski, M., Arnoult, D., Chen, H., Chan, D.C., Smith, C.L., and Youle, R.J., 2004a. Quantitation of mitochondrial dynamics by photolabeling of individual organelles shows that mitochondrial fusion is blocked during the Bax activation phase of apoptosis. J. Cell Biol. 164, 493-499.

Karbowski, M., Jeong, S.Y., and Youle, R.J., 2004b. Endophilin B1 is required for the maintenance of mitochondrial morphology. J. Cell Biol. 166, 1027-1039.

Karbowski, M., Lee, Y.J., Gaume, B., Jeong, S.Y., Frank, S., Nechushtan, A., Santel, A., Fuller, M., Smith, C.L., and Youle, R.J., 2002. Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis. J. Cell Biol. 159, 931-938.

ACCEPTED MANUSCRIPT

Karbowski, M., Norris, K.L., Cleland, M.M., Jeong, S.Y., and Youle, R.J., 2006. Role of Bax and Bak in mitochondrial morphogenesis. Nature 443, 658-662.

Kim, H., Tu, H.C., Ren, D., Takeuchi, O., Jeffers, J.R., Zambetti, G.P., Hsieh, J.J., and Cheng, E.H., 2009. Stepwise activation of BAX and BAK by tBID, BIM, and PUMA initiates mitochondrial apoptosis. Molecular Cell 36, 487-99.

Korsmeyer, S.J., Wei, M.C., Saito, M., Weiler, S., Oh, K.J., and Schlesinger, P.H., 2000. Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome c. Cell Death Differ. 7, 1166-1173.

Koshiba, T., Detmer, S.A., Kaiser, J.T., Chen, H., McCaffery, J.M., and Chan, D.C., 2004. Structural basis of mitochondrial tethering by mitofusin complexes. Science 305, 858-862.

Kuwana, T., Bouchier-Hayes, L., Chipuk, J.E., Bonzon, C., Sullivan, B.A., Green, D.R., and Newmeyer, D.D., 2005. BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. Molecular Cell 17, 525-535.

Kuwana, T., and Newmeyer, D.D., 2003. Bcl-2-family proteins and the role of mitochondria in apoptosis. Curr. Opin. Cell Biol. 15, 691-699.

Labi, V., Erlacher, M., Kiessling, S., and Villunger, A., 2006. BH3-only proteins in cell death initiation, malignant disease and anticancer therapy. Cell Death Differ. 13, 1325-1338.

Leber, B., Lin, J., and Andrews, D.W., 2007. Embedded together: the life and death consequences of interaction of the Bcl-2 family with membranes. Apoptosis 12, 897-911.

Lee, Y.J., Jeong, S.Y., Karbowski, M., Smith, C.L., and Youle, R.J., 2004. Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. Mol. Biol. Cell 15, 5001-5011.

Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S.M., Ahmad, M., Alnemri, E.S., and Wang, X., 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91, 479-89.

ACCEPTED MANUSCRIPT

Li, H., Zhu, H., Xu, C.J., and Yuan, J., 1998. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 94, 491-501.

Li, H., Chen, Y., Jones, A.F., Sanger, R.H., Collis, L.P., Flannery, R., McNay, E.C., Yu, T., Schwarzenbacher, R., Bossy, B., Bossy-Wetzel, E., Bennett, M.V., Pypaert, M., Hickman, J.A., Smith, P.J., Hardwick, J.M., and Jonas, E.A., 2008. Bcl-xL induces Drp1-dependent synapse formation in cultured hippocampal neurons. Proc. Natl. Acad. Sci. U S A 105, 2169-2174.

Liu, Q.A., and Shio, H., 2008. Mitochondrial morphogenesis, dendrite development, and synapse formation in cerebellum require both Bcl-w and the glutamate receptor delta2. PLoS Genetics 4, e1000097.

Logue, S.E., and Martin, S.J., 2008. Caspase activation cascades in apoptosis. Biochem. Soc. Trans. 36, 1-9.

Loucks, F.A., Schroeder, E.K., Zommer, A.E., Hilger, S., Kelsey, N.A., Bouchard, R.J., Blackstone, C., Brewster, J.L., and Linseman, D.A., 2009. Caspases indirectly regulate cleavage of the mitochondrial fusion GTPase OPA1 in neurons undergoing apoptosis. Brain Research 1250, 63-74.

Luthi, A.U., and Martin, S.J., 2007. The CASBAH: a searchable database of caspase substrates. Cell Death Differ. 14, 641-650.

Merino, D., Giam, M., Hughes, P.D., Siggs, O.M., Heger, K., O'Reilly, L.A., Adams, J.M., Strasser, A., Lee, E.F., Fairlie, W.D., and Bouillet, P., 2009. The role of BH3-only protein Bim extends beyond inhibiting Bcl-2-like prosurvival proteins. J Cell Biol 186, 355-62.

Narendra, D., Tanaka, A., Suen, D.F., Youle, R.J., Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. 2008. J Cell Biol. 183, 795-803.

Neuspiel, M., Zunino, R., Gangaraju, S., Rippstein, P., and McBride, H., 2005. Activated mitofusin 2 signals mitochondrial fusion, interferes with Bax activation, and reduces susceptibility to radical induced depolarization. J. Biol. Chem. 280, 25060-25070.

Niemann, A., Ruegg, M., La Padula, V., Schenone, A., and Suter, U., 2005.

ACCEPTED MANUSCRIPT

Ganglioside-induced differentiation associated protein 1 is a regulator of the mitochondrial network: new implications for Charcot-Marie-Tooth disease. J. Cell Biol. 170, 1067-1078.

Norris, K.L., and Youle, R.J., 2008. Cytomegalovirus proteins vMIA and m38.5 link mitochondrial morphogenesis to Bcl-2 family proteins. J. Virol. 82, 6232-6243.

Okamoto, K., and Shaw, J.M., 2005. Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. Annu. Rev. Genet. 39, 503-536.

Olichon, A., Baricault, L., Gas, N., Guillou, E., Valette, A., Belenguer, P., and Lenaers, G., 2003. Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. J. Biol. Chem. 278, 7743-7746.

Olichon, A., Emorine, L.J., Descoins, E., Pelloquin, L., Brichese, L., Gas, N., Guillou, E., Delettre, C., Valette, A., Hamel, C.P., Ducommun, B., Lenaers, G., and Belenguer, P., 2002. The human dynamin-related protein OPA1 is anchored to the mitochondrial inner membrane facing the inter-membrane space. FEBS Letters 523, 171-176.

Ow, Y.P., Green, D.R., Hao, Z., and Mak, T.W., 2008. Cytochrome c: functions beyond respiration. Nature Reviews Mol. Cell Biol. 9, 532-542.

Parone, P.A., James, D.I., Da Cruz, S., Mattenberger, Y., Donze, O., Barja, F., and Martinou, J.C., 2006. Inhibiting the mitochondrial fission machinery does not prevent Bax/Bak-dependent apoptosis. Mol. Cell Biol. 26, 7397-7408.

Ricci, J.E., Munoz-Pinedo, C., Fitzgerald, P., Bailly-Maitre, B., Perkins, G.A., Yadava, N., Scheffler, I.E., Ellisman, M.H., and Green, D.R., 2004. Disruption of mitochondrial function during apoptosis is mediated by caspase cleavage of the p75 subunit of complex I of the electron transport chain. Cell 117, 773-786.

Rolland, S.G., Lu, Y., David, C.N., and Conradt, B., 2009. The BCL-2-like protein CED-9 of C. elegans promotes FZO-1/Mfn1,2- and EAT-3/Opa1-dependent mitochondrial fusion. J. Cell Biol. 186, 525-540.

Santel, A., Frank, S., Gaume, B., Herrler, M., Youle, R.J., and Fuller, M.T., 2003. Mitofusin-1 protein is a generally expressed mediator of mitochondrial fusion in

ACCEPTED MANUSCRIPT

mammalian cells. J. Cell Sci. 116, 2763-2774.

Santel, A., and Fuller, M.T., 2001. Control of mitochondrial morphology by a human mitofusin. J. Cell Sci. 114, 867-874.

Sevrioukov, E.A., Burr, J., Huang, E.W., Assi, H.H., Monserrate, J.P., Purves, D.C., Wu, J.N., Song, E.J., and Brachmann, C.B., 2007. Drosophila Bcl-2 proteins participate in stress-induced apoptosis, but are not required for normal development. Genesis 45, 184-193.

Sheridan, C., Delivani, P., Cullen, S.P., and Martin, S.J., 2008. Bax- or Bak-induced mitochondrial fission can be uncoupled from cytochrome C release. Molecular Cell 31, 570-585.

Slee, E.A., Harte, M.T., Kluck, R.M., Wolf, B.B., Casiano, C.A., Newmeyer, D.D., Wang, H.G., Reed, J.C., Nicholson, D.W., Alnemri, E.S., Green, D.R., and Martin, S.J., 1999. Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. J. Cell Biol. 144, 281-292.

Smirnova, E., Griparic, L., Shurland, D.L., and van der Bliek, A.M., 2001. Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. Mol. Biol. Cell 12, 2245-2256.

Smirnova, E., Shurland, D.L., Ryazantsev, S.N., and van der Bliek, A.M., 1998. A human dynamin-related protein controls the distribution of mitochondria. J. Cell Biol. 143, 351-358.

Su, B., Wang, X., Zheng, L., Perry, G., Smith, M.A., and Zhu, X., 2010. Abnormal mitochondrial dynamics and neurodegenerative diseases. Biochim Biophys Acta. 1802,135-42.

Sugioka, R., Shimizu, S., and Tsujimoto, Y., 2004. Fzo1, a protein involved in mitochondrial fusion, inhibits apoptosis. J. Biol. Chem. 279, 52726-52734.

Sun, M.G., Williams, J., Munoz-Pinedo, C., Perkins, G.A., Brown, J.M., Ellisman, M.H., Green, D.R., and Frey, T.G., 2007. Correlated three-dimensional light and electron microscopy reveals transformation of mitochondria during apoptosis. Nature Cell Biology 9, 1057-1065.

Sutton, V.R., Davis, J.E., Cancilla, M., Johnstone, R.W., Ruefli, A.A., Sedelies,

ACCEPTED MANUSCRIPT

K., Browne, K.A., and Trapani, J.A., 2000. Initiation of apoptosis by granzyme B requires direct cleavage of bid, but not direct granzyme B-mediated caspase activation. J Exp Med 192, 1403-14.

Szabadkai, G., Simoni, A.M., Chami, M., Wieckowski, M.R., Youle, R.J., and Rizzuto, R., 2004. Drp-1-dependent division of the mitochondrial network blocks intraorganellar Ca2+ waves and protects against Ca2+-mediated apoptosis. Molecular Cell 16, 59-68.

Taguchi, N., Ishihara, N., Jofuku, A., Oka, T., and Mihara, K., 2007. Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. J. Biol. Chem 282, 11521-11529.

Tan, F.J., Husain, M., Manlandro, C.M., Koppenol, M., Fire, A.Z., and Hill, R.B., 2008. CED-9 and mitochondrial homeostasis in C. elegans muscle. J. Cell Sci. 121, 3373-3382.

Taylor, R.C., Cullen, S.P., and Martin, S.J., 2008. Apoptosis: controlled demolition at the cellular level. Nature Reviews Mol. Cell Biol. 9, 231-241.

Tondera, D., Santel, A., Schwarzer, R., Dames, S., Giese, K., Klippel, A., and Kaufmann, J., 2004. Knockdown of MTP18, a novel phosphatidylinositol 3-kinase-dependent protein, affects mitochondrial morphology and induces apoptosis. J. Biol. Chem. 279, 31544-31555.

Verhoeven, K., Claeys, K.G., Zuchner, S., Schroder, J.M., Weis, J., Ceuterick, C., Jordanova, A., Nelis, E., De Vriendt, E., Van Hul, M., Seeman, P., Mazanec, R., Saifi, G.M., Szigeti, K., Mancias, P., Butler, I.J., Kochanski, A., Ryniewicz, B., De Bleecker, J., Van den Bergh, P., Verellen, C., Van Coster, R., Goemans, N., Auer-Grumbach, M., Robberecht, W., Milic Rasic, V., Nevo, Y., Tournev, I., Guergueltcheva, V., Roelens, F., Vieregge, P., Vinci, P., Moreno, M.T., Christen, H.J., Shy, M.E., Lupski, J.R., Vance, J.M., De Jonghe, P., and Timmerman, V., 2006. MFN2 mutation distribution and genotype/phenotype correlation in Charcot-Marie-Tooth type 2. Brain 129, 2093-2102.

Wang, X., and Schwarz, T.L., 2009. The mechanism of Ca2+ -dependent regulation of kinesin-mediated mitochondrial motility. Cell 136, 163-174.

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Wasiak, S., Zunino, R., and McBride, H.M., 2007. Bax/Bak promote sumoylation of DRP1 and its stable association with mitochondria during apoptotic cell death. J. Cell Biol. 177, 439-450.

Wei, M.C., Zong, W.X., Cheng, E.H., Lindsten, T., Panoutsakopoulou, V., Ross, A.J., Roth, K.A., MacGregor, G.R., Thompson, C.B., and Korsmeyer, S.J., 2001. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 292, 727-730.

Yamaguchi, R., Lartigue, L., Perkins, G., Scott, R.T., Dixit, A., Kushnareva, Y., Kuwana, T., Ellisman, M.H., and Newmeyer, D.D., 2008. Opa1-mediated cristae opening is Bax/Bak and BH3 dependent, required for apoptosis, and independent of Bak oligomerization. Molecular Cell 31, 557-569.

Youle, R.J., and Strasser, A., 2008. The BCL-2 protein family: opposing activities that mediate cell death. Nature Reviews Mol. Cell Biol. 9, 47-59.

Yu, T., Fox, R.J., Burwell, L.S., and Yoon, Y., 2005. Regulation of mitochondrial fission and apoptosis by the mitochondrial outer membrane protein hFis1. J. Cell Sci. 118, 4141-4151.

Zuchner, S., Mersiyanova, I.V., Muglia, M., Bissar-Tadmouri, N., Rochelle, J., Dadali, E.L., Zappia, M., Nelis, E., Patitucci, A., Senderek, J., Parman, Y., Evgrafov, O., Jonghe, P.D., Takahashi, Y., Tsuji, S., Pericak-Vance, M.A., Quattrone, A., Battaloglu, E., Polyakov, A.V., Timmerman, V., Schroder, J.M., and Vance, J.M., 2004. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. Nature Genetics 36, 449-451.

Zunino, R., Schauss, A., Rippstein, P., Andrade-Navarro, M., and McBride, H.M., 2007. The SUMO protease SENP5 is required to maintain mitochondrial morphology and function. J. Cell Sci. 120, 1178-1188.

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Figure Legends

Fig 1. Bcl-2 family members regulate mitochondrial outer membrane permeabilization during apoptosis. There are three routes to caspase activation during apoptosis: the intrinsic, extrinsic and granzyme B pathways. The intrinsic pathway to apoptosis is initiated following extensive cellular stress or damage, which leads to the upregulation or activation of pro-apoptotic BH3-only proteins. BH3-only proteins then promote Bax/Bak-mediated pore formation in mitochondrial outer membranes, which facilitates the release of pro-apoptotic factors such as cytochrome *c* from mitochondria and downstream caspase activation. In healthy cells, anti-apoptotic Bcl-2 family members prevent mitochondrial outer membrane permeabilisation through inhibitory interactions with both BH3-only proteins and Bax/Bak. Granzyme B and death receptor-activated caspase-8 promote apoptosis through direct cleavage of caspase-3 and also through cleavage of the BH3-only protein Bid. This enhances Bid-mediated activation of Bax and Bak and promotes the mitochondrial pathway to apoptosis.

Fig 2. Mitochondrial fission and fusion. Mitochondrial fission is driven by Drp1 which resides primarily in the cytoplasm. Drp1 is recruited to mitochondria by a mechanism which is not fully understood but may involve Fis1 in some species. Drp1 then forms spirals around mitochondria at fission sites which promotes the constriction of mitochondria followed by fission. Fusion is driven by Mfn1 and Mfn2 localised on mitochondrial outer membranes. Interactions between these proteins tethers two adjacent mitochondria together and mitofusins then mediate mitochondrial outer membrane fusion while Opa1 mediates mitochondrial inner membrane fusion. During apoptosis, activation of Bax and Bak leads to the formation of pores in mitochondrial outer membranes resulting in the release of inner mitochondrial proteins such as cytochrome *c*. Activation of Bax and Bak also leads to mitochondrial fission. However, while anti-apoptotic Bcl-2 family

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members inhibit MOMP, they are unable to prevent mitochondrial fission indicating that these are distinct and separable events.

Fig 3. Mitochondrial fission is associated with apoptosis. Mitochondrial networks in HeLa cells transfected with a mitochondrially targeted green fluorescent protein (mito-GFP) plasmid (to visualise mitochondrial networks). Cells were left untreated (A) or were treated with the pro-apoptotic drug Actinomycin D (B).

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Table 1. Summary of the effects of the fission and fusion machinery on cytochrome *c* release and apoptosis

Method used to manipulate fission and fusion	**Effect on cytochrome *c* release and apoptosis**	**Reference**
Overexpression of Drp1 K38A (dominant negative)	Reduced cytochrome *c* release and protected against apoptosis Inhibited cytochrome *c* release and caspase activation triggered by truncated BAP31 overexpression Inhibited apoptosis triggered by azide treatment Reduced cytochrome *c* release triggered by BH3-only protein overexpression No effect on apoptosis No effect on cytochrome c release or apoptosis in response to a number of apoptotic drugs and Bim overexpression	Frank et al., 2001 Breckenridge et al., 2003 Brooks et al., 2007 Germain et al., 2005 Parone et al., 2006 Sheridan et al., 2008
Drp1 siRNA	Delays apoptosis Reduced cytochrome c release but did not affect smac release or apoptosis Partially prevented release of cytochrome c, but not other proteins, from mitochondria during apoptosis	Lee et al., 2004 Parone et al., 2006 Estaquier et al., 2007
Fis1 overexpression	No sensitisation to apoptosis using a number of apoptotic drugs	Sheridan et al., 2008
Fis1 siRNA	Reduces Bax translocation to mitochondria and apoptosis Partially reduced cytochrome c release from mitochondria but did not affect smac release or apoptosis	Lee et al., 2001 Parone et al., 2006
Mfn2 overexpression Mfn1 or Mfn2 overexpression	Reduced cytochrome c release in response to staurosorine treatment No effect on cytochrome c release or apoptosis in response to a number of apoptotic drugs and Bim overexpression	Neuspiel et al., 2005 Sheridan et al., 2008
Mfn1 siRNA	No effect on cytochrome c release from isolated mitochondria when treated with tBid	Arnoult et al., 2005a
Opa1 overexpression	Did not reduce cytochrome c release or apoptosis in response to a number of apoptotic drugs	Sheridan et al., 2008
Opa1 siRNA	Sensitises cells to apoptosis Cytochrome c is more rapidly released from isolated mitochondria when treated with tBid Knockdown of Opa1 induces apoptosis that can be inhibited by Bcl-2	Lee et al., 2001 Arnoult et al., 2005a Olichon et al., 2003

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Figure 1

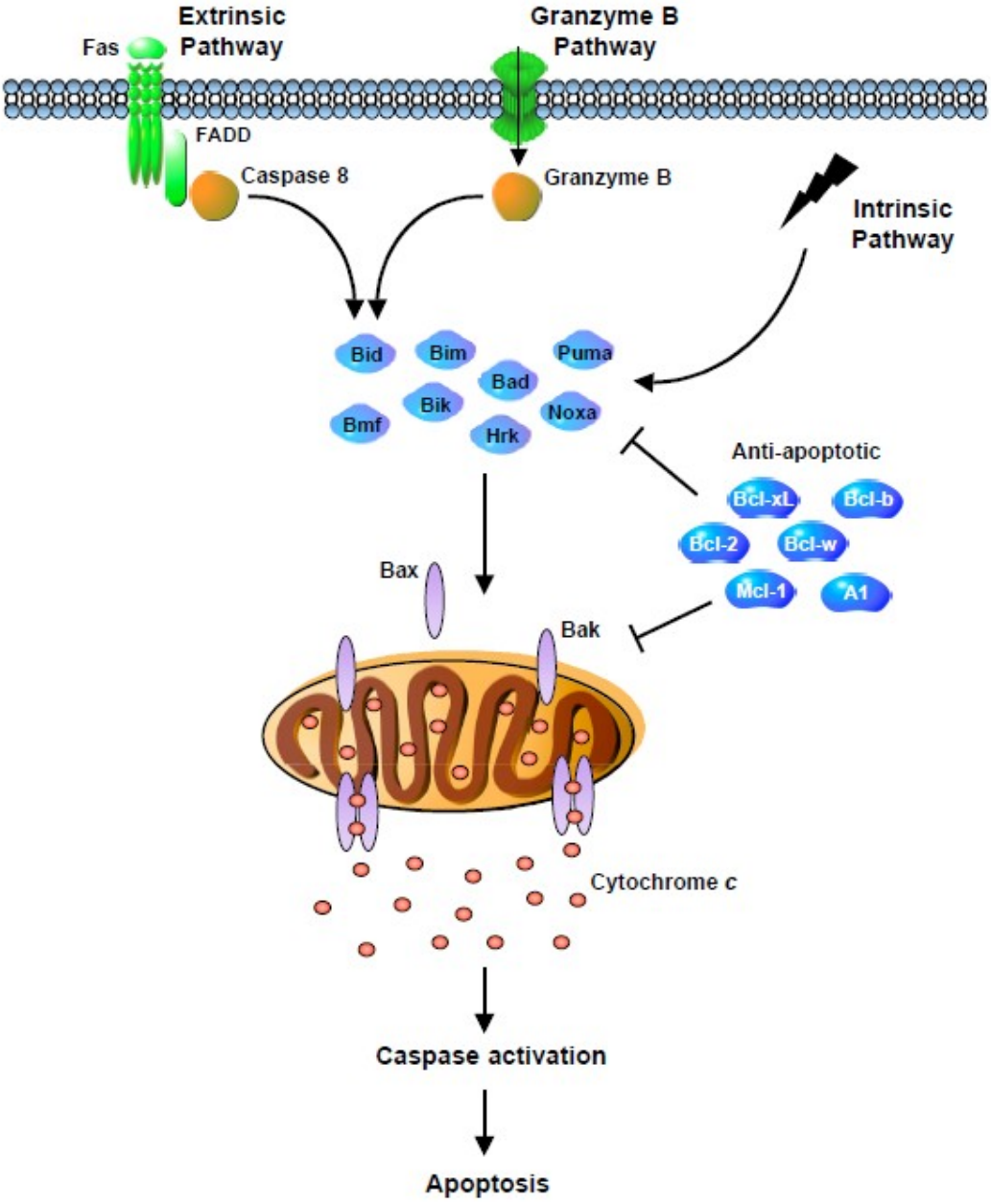

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Figure 2

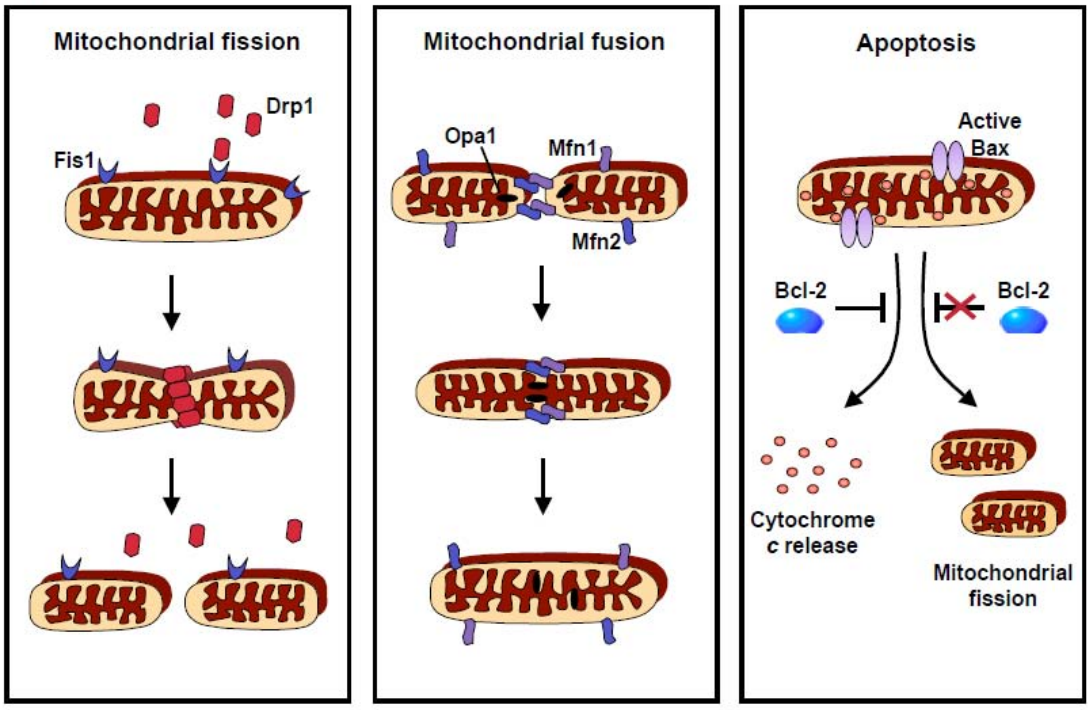

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Figure 3