

# Emerging Role for Members of the Bcl-2 Family in Mitochondrial Morphogenesis

Arnaud Autret<sup>1</sup> and Seamus J. Martin<sup>1,\*</sup>

<sup>1</sup>Molecular Cell Biology Laboratory, Department of Genetics, The Smurfit Institute, Trinity College, Dublin 2, Ireland

\*Correspondence: [martinsj@tcd.ie](mailto:martinsj@tcd.ie)

DOI 10.1016/j.molcel.2009.10.011

**Bcl-2 family proteins regulate apoptosis by controlling the release of mitochondrial cytochrome *c* via the Bax/Bak channel. However, recent studies have also implicated several members of this family in the regulation of mitochondrial fission/fusion dynamics. It has been debated whether the role of Bcl-2 proteins in mitochondrial morphogenesis is functionally distinct from their role in apoptosis, with some arguing that Bax/Bak-induced mitochondrial fission promotes apoptosis-associated cytochrome *c* release, while others suggest that these functions are separable. Here we review this emerging area and argue for a role for the Bcl-2 family as novel regulators of mitochondrial morphogenesis.**

## Introduction

Apoptosis is a mode of programmed cell death that is used in a diversity of settings to eliminate cells that are only transiently required, are injured, are infected, or have reached the end of their useful life span. This mode of cell death is coordinated by members of a family of proteases—the caspases—that are normally present as inactive precursors in healthy cells but become activated at the onset of apoptosis. Upon activation, caspases unleash controlled mayhem within the cell, involving the restricted proteolysis of hundreds of substrate proteins (reviewed in [Luthi and Martin, 2007](#)). The latter events culminate in cell death with the morphological and biochemical features of apoptosis ([Taylor et al., 2008](#)). Programmed elimination of cells via apoptosis ensures the timely and controlled removal of cellular corpses before such cells can rupture and release their contents. The latter feature is of critical importance, as cell rupture (i.e., necrosis) can provoke inflammatory immune reactions that escalate damage and lead to further cell death ([Chen et al., 2007](#); [Kono and Rock, 2008](#)).

Members of the Bcl-2 protein family play pivotal roles in regulating caspase activation through regulating mitochondrial outer membrane permeability (MOMP). This is because one of the major routes to apoptosis-associated caspase activation—the so-called intrinsic pathway to apoptosis—is initiated upon release of cytochrome *c* from the mitochondrial intermembrane space into the cytosol (reviewed in [Taylor et al., 2008](#)). Upon entering the cytosol, cytochrome *c* acts as a cofactor for a caspase-activating molecule, called Apaf-1, which is normally present in an autoinhibited state within healthy cells. Upon binding cytochrome *c*, Apaf-1 undergoes oligomerization into a disc-like complex—termed the apoptosome—and this acts as an activation platform for caspase-9 ([Youle and Strasser, 2008](#); [Taylor et al., 2008](#)). Upon activation within the apoptosome, caspase-9 then initiates a cascade of further caspase activation events that result in apoptosis ([Figure 1](#)).

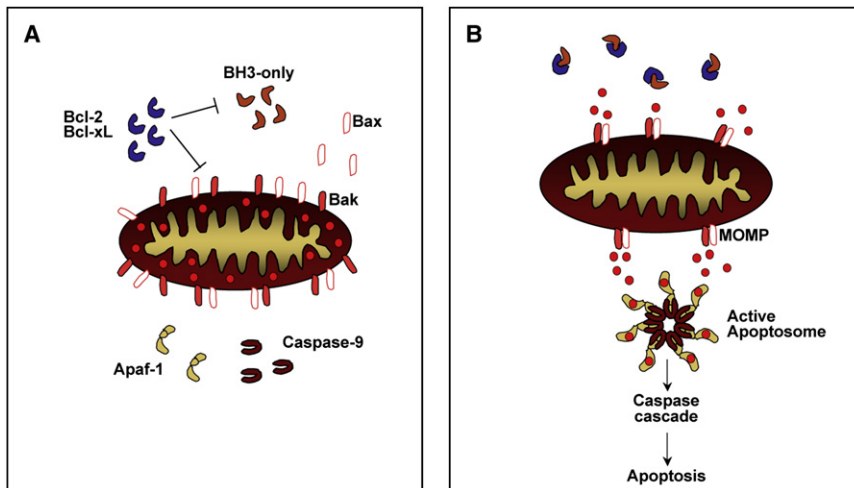
Thus, cells use cytosolic cytochrome *c* as a cue to activate the programmed cell death machinery. Because the decision to initiate apoptosis is such an important one in the life of a cell, a decision that can be made only once, it follows that cyto-

chrome *c* must be under a tight leash within the mitochondrial intermembrane space, lest this molecule inadvertently escape and provoke death.

## Bcl-2 Family Proteins Regulate MOMP

Aside from the hazards of apoptosome activation, the permeability of the outer mitochondrial membrane is also tightly regulated to ensure that mitochondrial respiratory chain components, including cytochrome *c*, do not leak out into the cytosol and impair ATP production as a consequence. Indeed, maintenance of optimal oxidative phosphorylation-dependent ATP production is essential for viability of many cell types. As an illustration of this, when cytochrome *c*-dependent caspase activation is abolished as a result of inactivation of the genes encoding Apaf-1 or caspase-9, MOMP typically leads to caspase-independent cell death in most cell types ([Ekert et al., 2004](#)). This is due to a precipitous decline in ATP production and mitochondrial dysfunction as a result of the escape of mitochondrial intermembrane space constituents into the cytosol ([Mootha et al., 2001](#); [Colell et al., 2007](#)). Because of this, MOMP is a defining event in the life of a cell, a point at which commitment to cell death becomes irreversible even if caspases fail to be activated downstream. It should be noted, however, that cell death without caspase activation lacks the majority, if not all, of the features of apoptosis and is otherwise indistinguishable from necrosis ([Kroemer and Martin, 2005](#)). Irrespective of whether death occurs via apoptosis or necrosis, MOMP is undoubtedly bad news for a cell and must be carefully policed, which is where the Bcl-2 family proteins arrive center stage.

Apoptosis-associated MOMP is known to require Bax and/or Bak, two closely related members of the Bcl-2 family that reside on the mitochondrial outer membrane or translocate there in response to proapoptotic stimuli ([Wei et al., 2001](#); [Wolter et al., 1997](#); [Youle and Strasser, 2008](#)). Normally, Bax and Bak are present in cells as inactive monomers, possibly held in this state by antiapoptotic members of the Bcl-2 family, such as Bcl-2 itself, and become activated during apoptosis via the actions of one or more members of the BH3-only subset of the Bcl-2 family ([Youle and Strasser, 2008](#); [Figure 1](#)).



**Figure 1. Bcl-2 Family Proteins Regulate Mitochondrial Outer Membrane Permeabilization**

(A) In viable cells, members of the antiapoptotic subset of the Bcl-2 family (Bcl-2, Bcl-xL, A1, Mcl-1, Bcl-w, Bcl-b) inhibit the activity of the BH3-only proteins (Bad, Bid, Bim, Bik, Bmf, Noxa, Puma, Hrk) and prevent the activation of Bax and Bak.

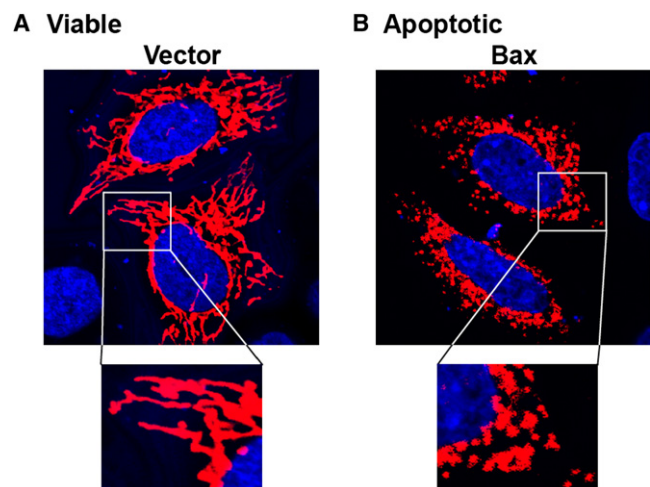
(B) Upon activation of one or more members of the BH3-only subset of the Bcl-2 family, these proteins promote MOMP and consequent cytochrome c release through neutralizing the antiapoptotic Bcl-2 proteins and/or triggering Bax/Bak oligomerization within the mitochondrial outer membrane. Upon release of cytochrome c into the cytosol, this promotes assembly of the Apaf-1/caspase-9 apoptosome and triggers a cascade of caspase activation events that culminate in apoptosis.

BH3-only proteins, such as Bid, Bim, and Bad, are the most upstream acting members of the Bcl-2 family and have little in common with each other, or indeed with other members of this family, except for an ~8 amino acid long BH3-only motif. BH3-only proteins act as sensors for cell stress, damage, infection, growth factor deprivation, and other signals that provoke apoptosis (Puthalakath and Strasser, 2002). The BH3-only sensors become activated by a variety of means, such as transcriptional upregulation, limited proteolysis, or dephosphorylation. Irrespective of how particular BH3-only proteins are activated, these proteins promote Bax/Bak activation by two possible mechanisms, which are not mutually exclusive. One route to Bax/Bak activation involves displacement of the antiapoptotic Bcl-2 proteins from Bax/Bak due to binding of BH3-only proteins to the antiapoptotic proteins (Willis et al., 2007). Another possible route to Bax/Bak activation is via direct, but possibly transient, interaction of BH3-only proteins with Bax/Bak that provokes a conformational change of these proteins to their active state (Kuwana et al., 2005).

Whichever model is correct—noting that both mechanisms could well operate simultaneously—Bax/Bak activation results in the formation of a pore or channel in the mitochondrial outer membrane that permits release of multiple mitochondrial proteins into the cytosol (Figure 1). Aside from cytochrome c, MOMP is also associated with efflux of Smac/DIABLO, a potentiator of caspase activation, as well as Omi/HtrA2, adenylate kinase 2, and many other mitochondrial intermembrane space proteins. As noted above, this event has catastrophic consequences for the cell, due to the swift activation of the Apaf-1/caspase-9 apoptosome upon binding of cytochrome c. Because of the importance of this event for commitment to apoptosis, Bax/Bak-dependent cytochrome c release has been studied intensively over the past 10 years. During the course of such studies, it was also noticed that mitochondria, which form extensive interconnected networks in many cells, undergo extensive fragmentation practically simultaneously with apoptosis-associated mitochondrial cytochrome c release (Frank et al., 2001; Figure 2). Before we discuss the potential implications of this observation, we will first briefly discuss mitochondrial network dynamics in healthy cells.

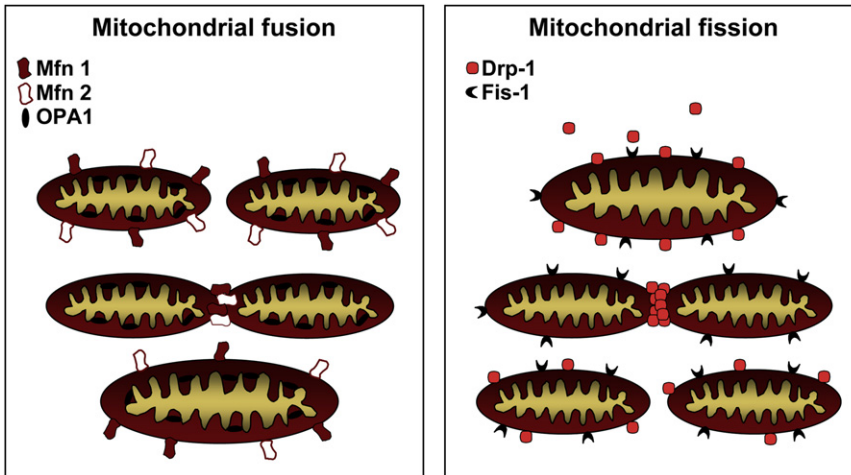
### Mitochondrial Fission and Fusion

Contrary to the classical textbook view that mitochondria are small bean-shaped organelles scattered throughout the cytosol, many cells contain long tubular mitochondria that are extensively interconnected to form web-like networks that encompass the whole cell (Figure 2A). Mitochondrial networks are highly dynamic and undergo remodeling through continuous cycles of fission and fusion to produce shorter or longer mitochondria (Chen and Chan, 2005; Suen et al., 2008). Indeed, experiments where cells with differentially labeled populations of mitochondria have been induced to undergo fusion indicate that intermixing of mitochondrial constituents is essentially complete within 10–12 hr (Legros et al., 2002). Furthermore, mitochondrial networks can undergo abrupt remodeling in response to cell stress (Tondera et al., 2009), changes in energy demands, and fluctuations in intracellular calcium levels



**Figure 2. Mitochondrial Networks Undergo Fragmentation during Apoptosis**

Mitochondrial networks in HeLa cells transfected with a mitochondrially targeted red fluorescent protein (mito-RFP) plasmid (to visualize mitochondrial networks) along with an empty vector plasmid (A) or Bax-expressing plasmid (B).



**Figure 3. Molecular Control of Mitochondrial Fission and Fusion**

Proteins implicated in mitochondrial fusion (left panel) or fission (right panel). (Left panel) The mitochondrial outer membrane proteins, Mfn1 and Mfn2, in association with the inner membrane protein Opa1, promote fusion of outer as well as inner mitochondrial membranes to produce longer mitochondria. (Right panel) Drp1 forms oligomers encircling mitochondria that might function through progressively constricting mitochondria to form separate organelles. It is not clear precisely how Fis1 operates in higher eukaryotes, but this protein might cooperate with Drp1 to promote mitochondrial fission.

(Szabadkai et al., 2004) and in preparation for mitosis (Mitra et al., 2009).

Unsurprisingly, mitochondrial fission and fusion are highly regulated and serve several purposes, including ensuring equal partitioning of mitochondria to daughter cells during mitosis, mitochondrial replication, repair of defective mitochondria, and propagation of calcium waves (Chan, 2006). Furthermore, the state of the mitochondrial network may also influence the propensity of cells to enter apoptosis, as we shall discuss in more detail below.

Mitochondrial fusion is controlled by a cohort of proteins that regulate the tethering of adjacent mitochondria, followed by fusion of their inner and outer membranes (Figure 3). Although the detailed mechanics of mitochondrial fusion are still not understood, it is well established that mitofusins (Mfn) 1 and 2, dynamin family GTPases that engage in homo- as well as heterotypic interactions on adjacent mitochondria, play prominent roles in mitochondrial fusion (Chen and Chan, 2005; Chen et al., 2003). Mfn1 and Mfn2 are localized to mitochondrial outer membranes where regions within their C-termini are thought to interact, *in trans*, between neighboring mitochondria to promote tethering and fusion (Koshihara et al., 2004). Another dynamin family GTPase, Opa1, which is localized to the mitochondrial intermembrane space and the mitochondrial inner membrane, cooperates with the mitofusins in promoting mitochondrial fusion. Loss of Opa1, Mfn1, or Mfn2 leads to defective mitochondrial fusion and hence provokes fragmentation of the mitochondrial network to varying degrees (Chen et al., 2003; Frezza et al., 2006; Griparic et al., 2004).

Mitochondrial fission is also regulated by a GTPase, dynamin-related protein 1 (Drp1), that can form ring-like oligomers at specific points along mitochondria (Figure 3). Once again, the details remain to be worked out, but Drp1 oligomers may be capable of physically constricting and pinching mitochondria apart in a drawstring-like mechanism. Interestingly, much of the cellular Drp1 content is not constitutively associated with mitochondrial membranes but translocates to discrete foci on mitochondria that often become future sites of fission. Fis1, a protein that is uniformly dispersed on mitochondrial outer membranes, is also involved in mitochondrial fission and might

act as a receptor for Drp1 in yeast (Mozdy et al., 2000), although that has yet to be confirmed in higher eukaryotes. Indeed, Youle and colleagues have found that Fis1 knockdown in mammalian cells does not interfere with the translocation of Drp1 to mitochondria (Lee et al., 2004), suggesting either that Fis1 is redundant with other factors in mammals or that mammalian Fis1 is not a receptor for Drp1.

The importance of mitochondrial fission/fusion for cellular homeostasis is underscored by the phenotypes of mice mutant in many of the genes implicated as central players in mitochondrial fission/fusion. Inactivation of the genes encoding either Mfn1, Mfn2, Opa1, or Drp1 in the mouse are all lethal (Chen et al., 2003; Frezza et al., 2006; Ishihara et al., 2009), and mutations in several of these genes in humans are associated with neurodegenerative conditions, most likely as a direct result of perturbing mitochondrial morphogenesis in neurons (Chan, 2006). Neurons, as well as other highly polarized cells, appear to be particularly sensitive to disturbances in mitochondrial network dynamics, as such cells, due to their extreme size and polarity, often require localization of mitochondria at specific sites, such as axon terminals where synapses are formed. This may be due to the high ATP demands at such sites and/or the role of mitochondria in cellular calcium buffering. It has also been found that the branching processes of such cells, the dendritic spines and axonal terminals, form in close association with mitochondrial network outgrowth, most likely due to the local high energy demands within these cellular regions (Li et al., 2004). Irrespective of the precise role of mitochondria at such sites, defects in mitochondrial fission/fusion often manifest in neurodegenerative diseases such as optic atrophy and Charcot-Marie-Tooth disease type 2A (reviewed in Chan, 2006).

#### Mitochondrial Fission Is Associated with Apoptosis

Mitochondrial networks were first reported to undergo fragmentation during apoptosis almost 10 years ago (Desagher and Martinou, 2000; Frank et al., 2001). However, the fact that mitochondrial fission is often associated with cell injury has been known for over 50 years, well before the term “apoptosis” was even coined (Claude and Fullam, 1945). Before delving into the possible causes of apoptosis-associated mitochondrial

fragmentation, it should be said at the outset that opinion is divided about whether this phenomenon is simply a consequence of apoptosis or plays a more active role in the process. As we shall discuss below, some investigators have suggested that mitochondrial fission may promote cytochrome *c* release and therefore act to drive caspase activation during apoptosis (Frank et al., 2001; Youle and Karbowski, 2005). However, other data suggest that apoptosis-associated mitochondrial fission is a consequence rather than a cause of apoptosis and reflects events that impinge upon some hitherto unrecognized connection between members of the Bcl-2 family and the mitochondrial morphogenesis machinery (Arnoult et al., 2005; Delivani et al., 2006; Karbowski et al., 2006; Sheridan et al., 2008).

Irrespective of the functional role of apoptosis-associated mitochondrial fission, what is widely agreed upon is that this is a general feature of apoptosis in essentially all cell types and in response to most, if not all, proapoptotic stimuli (Frank et al., 2001; Karbowski et al., 2002; Arnoult et al., 2005; Sheridan et al., 2008). Not only do mitochondrial networks undergo fragmentation during apoptosis, but these fragmented mitochondria also become clustered around the nucleus, and all mitochondrial movement appears to cease (Sheridan et al., 2008; Figure 2B). It is not clear exactly how this happens, but the end result is that mitochondria within apoptotic cells appear to become uncoupled from their normal attachment points to the cytoskeleton, an observation that awaits further investigation.

### Bax and Bak Can Promote Mitochondrial Fission

So, how does mitochondrial fission/fragmentation occur during apoptosis? Youle and colleagues have published a series of seminal studies demonstrating conclusively that activation of Bax and/or Bak leads rapidly to mitochondrial fragmentation (Frank et al., 2001; Karbowski et al., 2002, 2004). Furthermore, this event occurs very close in time to MOMP and associated mitochondrial cytochrome *c* release (Arnoult et al., 2005). What is also highly compelling is that activated Bax—which can be distinguished from the inactive form of this protein using conformational-sensitive Bax antibodies—frequently decorates mitochondrial scission sites (Karbowski et al., 2004). Apoptosis-associated mitochondrial fragmentation occurs upstream of caspase activation, as inhibition of caspase activity downstream of Bax/Bak activation fails to block this event (Frank et al., 2001; Sheridan et al., 2008). These data have led to the hypothesis that Bax/Bak-dependent mitochondrial fragmentation contributes to the process of MOMP and promotes the release of cytochrome *c* and other intermembrane space constituents that is seen during apoptosis (Desagher and Martinou, 2000; Youle and Karbowski, 2005).

Some other observations support the idea that mitochondrial fragmentation can facilitate apoptosis-associated cytochrome *c* release. A dominant-negative mutant of Drp1 (Drp1<sup>K38A</sup>) that can impair mitochondrial fission has been observed to impair cytochrome *c* release and delay cell death in response to some proapoptotic agents (Frank et al., 2001; Brooks et al., 2007). However, it should be noted that the delay in cytochrome *c* release observed is relatively modest and has not been shown to translate into long-term survival. Indeed, *Drp1*<sup>-/-</sup> null mice exhibit elevated levels of apoptosis, arguing that this protein is

dispensable for MOMP (Ishihara et al., 2009). The delayed kinetics of cytochrome *c* release seen when Drp1 expression is ablated or functionally impaired may be a nonphysiological by-product of altered mitochondrial ultrastructure in such cells, because the release kinetics of Smac, Omi, adenylate kinase 2, and other intermembrane space proteins was unaltered under the same conditions (Estaquier and Arnoult, 2007; Parone et al., 2006; Ishihara et al., 2009). Because Smac and cytochrome *c* are coreleased in a Bax/Bak-dependent manner (Munoz-Pinedo et al., 2006), this suggests that cells defective in Drp1 may exhibit artificially delayed cytochrome *c* release kinetics. One possible reason for this is that remodeling of the cristae junctions in such cells—known to be required for efficient cytochrome *c* release (Scorrano et al., 2002)—may be affected. However, because the release kinetics of Smac, Omi, adenylate kinase 2, and other intermembrane space proteins is overtly normal, this suggests that MOMP is unaffected through loss of Drp1.

Observations that, at first sight, appear to be at odds with the latter view have been made by Nunnari and colleagues using a pharmacological inhibitor of Drp-1, mdivi-1 (Cassidy-Stone et al., 2008). This inhibitor, which interferes with mitochondrial fission in yeast and mammalian cells, has been found to attenuate cytochrome *c* release and apoptosis in response to staurosporine. However, mdivi-1 was also found to inhibit bid-dependent cytochrome *c* release from isolated mitochondria (Cassidy-Stone et al., 2008), which is puzzling, as purified mitochondria do not undergo fission *in vitro*. This suggests either that mdivi-1 is also capable of inhibiting other (unknown) molecules that regulate MOMP, or that Drp1 plays a role in MOMP that is unrelated to its role in mitochondrial fission. Either way, this study does not provide support for a simple connection between mitochondrial fission and MOMP.

Overexpression of Mfn2, which produces highly fused mitochondria, has been reported to delay cytochrome *c* release (Jahani-Asl et al., 2007). However, others find little impairment of apoptosis upon overexpression of Mfn1, Mfn2, or Drp1<sup>K38A</sup> (Sheridan et al., 2008), leading to the view that mitochondrial fission is unlikely to be essential for MOMP and apoptosis but acts as a contributory factor in the release of mitochondrial factors rather than a key driver of this process. So why does mitochondrial fragmentation invariably occur during apoptosis?

A plausible interpretation of the available data is that mitochondrial networks fragment during apoptosis because the events that open the Bax/Bak channel (i.e., activation of one or more BH3-only proteins) simultaneously perturb the function of members of this family involved in mitochondrial morphogenesis, as well as MOMP. Put another way, we propose that one or more members of the Bcl-2 family play roles in mitochondrial morphogenesis that are functionally distinct from their role as regulators of MOMP. During apoptosis, the events that promote MOMP also coincidentally affect the role that these Bcl-2 family members play in mitochondrial network dynamics, resulting in mitochondrial fission.

This proposal predicts that perturbing the Bcl-2 family network in healthy cells may result in changes in mitochondrial network dynamics, either through increased fusion or fission, in the absence of MOMP or apoptosis. Indeed, several studies have already provided evidence for this (Karbowski et al., 2006; Liu

and Shio, 2008; Sheridan et al., 2008; Tan et al., 2008; Berman et al., 2009). Another prediction is that it should be possible to provoke Bax/Bak-dependent mitochondrial fragmentation in healthy cells in the absence of MOMP. Once again, this prediction has also been fulfilled, as we shall discuss below. Furthermore, it is also expected that members of the Bcl-2 family may interact with core constituents of the mitochondrial fission/fusion machinery such as Drp1, Mfn1, or Mfn2. Such evidence has also recently emerged (Delivani et al., 2006; Brooks et al., 2007; Li et al., 2008).

### Bax/Bak-Induced Fission Can Be Uncoupled from Cytochrome c Release

The observation that Bax/Bak-induced mitochondrial fragmentation occurs almost simultaneously with the release of mitochondrial cytochrome c suggested that these events could be related. However, several independent observations suggest that these events are merely coincident and can be functionally uncoupled.

Karbowski and Youle have shown that Bax/Bak double knockout cells have constitutively fragmented mitochondrial networks, despite being highly resistant to MOMP and apoptosis (Karbowski et al., 2006). Re-expression of Bak in these cells, under conditions where apoptosis was not provoked, led to increases in mitochondrial length, arguing that Bax and/or Bak can promote mitochondrial fusion/elongation in the absence of apoptosis. These authors also presented evidence that Bax/Bak-induced mitochondrial fusion may occur via regulating Mfn2 function, although a direct interaction between these two proteins was not demonstrated in this study. These intriguing observations suggest that Bax and/or Bak contribute to mitochondrial fusion quite apart from their role as positive regulators of MOMP. In this light, mitochondrial fragmentation during apoptosis could be interpreted as a direct consequence of neutralization of the Bax/Bak-dependent mitochondrial fusion activity due to the conformational changes that the latter proteins undergo upon activation (Dewson et al., 2008).

Additional evidence for an apoptosis-independent role for Bax/Bak in mitochondrial fusion comes from two independent studies on the CMV viral protein, vMIA, which binds Bax and Bak and inhibits apoptosis (Karbowski et al., 2006; Roumier et al., 2006). Both studies reported that vMIA provokes mitochondrial fragmentation without either MOMP or apoptosis, once again suggesting that neutralization of Bax/Bak function disturbs the mitochondrial network in healthy cells, leading to mitochondrial fission in the absence of MOMP. However, it should be noted that there is also evidence to suggest that the effects of vMIA on mitochondrial fission-fusion dynamics may be Bax/Bak independent (Poncet et al., 2006).

Further evidence for an uncoupling of Bax/Bak-associated mitochondrial fission and MOMP comes from studies in which Bax or Bak was transiently expressed along with antiapoptotic members of the Bcl-2 family, such as Bcl-xL or Bcl-2 (Delivani et al., 2006; Sheridan et al., 2008). In all cases, Bcl-xL or Bcl-2 failed to suppress Bax-induced mitochondrial fission but readily suppressed MOMP and apoptosis downstream.

Collectively, these studies argue that perturbing the balance of interactions within the Bcl-2 family, even in situations in which

this does not lead to apoptosis, also impacts upon the connectivity of the mitochondrial network. These data argue that the Bcl-2 family may play a previously unsuspected role as regulators of mitochondrial morphogenesis, quite apart from their role in apoptosis. We will now take a look at further evidence for this.

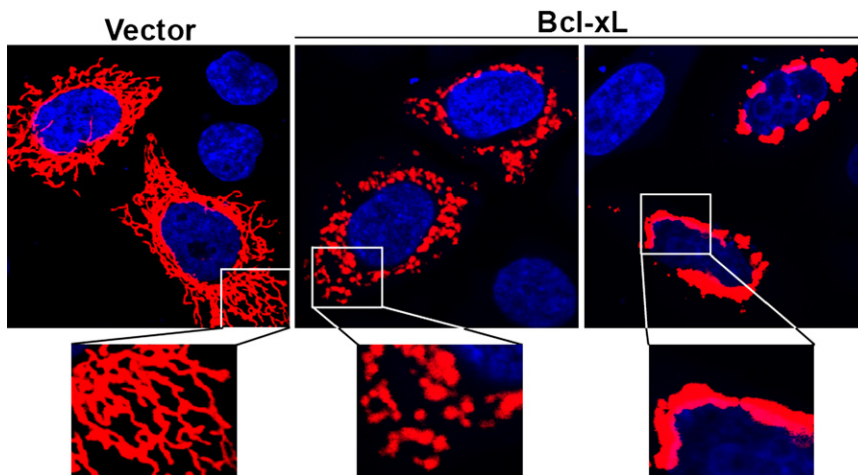
### Bcl-2 Family Members Can Remodel Mitochondrial Networks in Healthy Cells

Evidence from a number of laboratories is accumulating to suggest that members of the Bcl-2 family may influence mitochondrial network dynamics in healthy cells. Perhaps the most persuasive evidence comes from *Bcl-w* and *Bcl-xL* knockout mice, where defects in mitochondrial networks have recently been reported (Liu and Shio, 2008; Berman et al., 2009).

Using *Bcl-w*<sup>-/-</sup> animals, Liu and Shio have made the unexpected observation that mitochondria in cerebellar purkinje neurons are approximately 2-fold longer than normal in these animals (Liu and Shio, 2008). This suggests that Bcl-w may be required for normal rates of mitochondrial fission, although this was not directly measured in this study. The longer mitochondria seen in these animals may be due to normal rates of ongoing fusion, without counterbalancing fission, in the absence of Bcl-w. Somewhat surprisingly, the authors found little evidence that Bcl-w controls cell survival in the mouse, as cell numbers were overtly normal, but these mice did exhibit defective learning responses, which is very plausibly related to defective mitochondrial fission. This is because mitochondrial fission has been found to be required for normal synaptogenesis, as discussed earlier (Li et al., 2004).

Using conditional *Bcl-xL*<sup>-/-</sup> knockout mice, Hardwick and colleagues provide evidence that Bcl-xL can promote increased fission as well as fusion in healthy neurons. Mitochondria within healthy cortical and hippocampal neurons from *Bcl-xL*<sup>-/-</sup> animals were shorter and more punctiform than their wild-type counterparts (Berman et al., 2009). Furthermore, transient expression of Bcl-xL in wild-type rat cortical neurons increased mitochondrial lengths (Berman et al., 2009). The simple interpretation of these data is that Bcl-xL is required for mitochondrial fusion. However, through careful analysis of rates of both fission and fusion in the presence of overexpressed Bcl-xL, Hardwick and colleagues found that this protein increased rates of mitochondrial fusion as well as fission, and an effect on mitochondrial biomass was also noted (Berman et al., 2009).

An intriguing connection between Bcl-xL and neuronal synaptogenesis, reported by Jonas and colleagues (Li et al., 2008), may also reflect a role for Bcl-xL in the regulation of mitochondrial fission, rather than apoptosis. Li et al. found that Bcl-xL expression enhanced synapse formation and synaptic activity in neurons, an effect that could be abrogated by blocking mitochondrial fission with dominant-negative Drp1<sup>K38A</sup>. As noted earlier, this observation is consistent with the known role of mitochondrial fission in promoting synapse formation (Chan, 2006). Furthermore, a physical interaction between Bcl-xL and Drp1 was also reported in this study, and Bcl-xL was found to increase the GTPase activity of Drp-1 in vitro (Li et al., 2008). Therefore, by acting as a positive regulator of Drp1-dependent fission, Bcl-xL may influence the propensity of mitochondria to divide, which in



**Figure 4. Bcl-2 Family Proteins Can Perturb Mitochondrial Networks in Healthy Cells**

Mitochondrial networks in cells transfected with a mito-RFP plasmid along with empty vector plasmid (left panel) or Bcl-xL expression plasmid (middle and right panels). Bcl-xL expression results in cells with mitochondrial networks that are either fragmented (middle panel) or hyperaggregated and/or fused (right panel). See Delivani et al. (2006) and Sheridan et al. (2008) for further details.

turn may influence the availability of mitochondria for the formation of new branches at axon terminals and dendritic spines.

Previous studies have also implicated other members of the Bcl-2 family as regulators of mitochondrial morphogenesis. Perhaps the first report to explicitly draw attention to the possibility that Bcl-2 may affect normal mitochondrial morphology in healthy cells was a study by Fiskum and colleagues (Kowaltowski et al., 2002). Using flow cytometry to measure the size and granularity of isolated mitochondria, this report noted that Bcl-2-overexpressing cells contained mitochondria that were larger and more structurally complex than normal, as indicated by their greatly increased laser scattering properties.

Further evidence for a role for members of the Bcl-2 family in steady-state mitochondrial network dynamics has also been provided by studies in which Bcl-xL and Bcl-2 have been transiently expressed in a variety of mammalian cell types (Delivani et al., 2006; Sheridan et al., 2008). These studies reported that both Bcl-2 family proteins could promote increased rates of mitochondrial fusion or mitochondrial fission, depending on the concentration of expressed protein (Figure 4). In all cases, perturbation of mitochondrial network dynamics, through overexpression of Bcl-2 or Bcl-xL, led neither to MOMP nor to apoptosis. Furthermore, similar observations have been made with the *C. elegans* Bcl-2 homolog, CED-9, that has been found to promote increased mitochondrial fusion upon ectopic expression in either mammalian cells (Delivani et al., 2006), *C. elegans* embryos (Rolland et al., 2009), or mature worm muscle tissue (Li et al., 2008; Rolland et al., 2009).

#### Mitochondrial Network Connectivity May Exert Long-Range Effects on the Threshold for MOMP

It may seem odd, at first sight, that members of the Bcl-2 family could impinge on the spatial organization of mitochondrial networks, given the overwhelming body of evidence that places this protein family firmly within the heart of the cell death machinery. However, these seemingly disparate functions may not be entirely unconnected.

Accumulating evidence indicates that the efficiency of oxidative phosphorylation within the mitochondrial electron transport chain is affected by the degree of connectivity of mitochondria,

with highly connected mitochondria correlating with increased efficiency of ATP production (Chen et al., 2005; Mitra et al., 2009; Tondera et al., 2009). For example, fragmentation of mitochondrial networks, through genetic inactivation of *Mfn1* and *Mfn2* or RNAi-mediated ablation of *Opa1*, led to markedly decreased rates of respiration (Chen et al., 2005). These defects could be reversed through restoration of mitochondrial fusion, suggesting that these defects were directly related to fusion incompetence and not due to a loss of mitochondrial DNA or other long-range effects (Chen et al., 2005).

Conversely, cells under starvation stress have been observed to increase mitochondrial connectivity and ATP production, which required *Mfn-1* (Tondera et al., 2009), presumably as an adaptive response to the change in nutrient availability. Where stress-induced mitochondrial fusion was prevented, in *Mfn1* null cells, for example, ATP production declined and cells died more rapidly (Tondera et al., 2009). On the other hand, where mitochondrial connectivity was restored through overexpression of *Mfn-1*, cell survival was enhanced.

However, the relationship between mitochondrial network status and respiratory function is clearly not a simple one, as knockdown of *Drp1*, which leads to mitochondrial elongation, can also lead to defective respiration (Estaquier and Arnoult, 2007; Benard et al., 2007; Parone et al., 2008), although *Drp1*<sup>-/-</sup> MEFs do not appear to suffer from such defects (Ishihara et al., 2009). However, these studies do provide further support for the view that mitochondrial interconnectivity can influence ATP production in either a positive or a negative way.

Given the role of the antiapoptotic members of the Bcl-2 family in cell survival, it is possible that, in addition to regulating Bax/Bak channel formation and apoptosis directly, these proteins may also be capable of regulating conformations of the mitochondrial network that favor cell survival by enhancing ATP synthesis and raising the threshold for MOMP. We suggest that while fragmentation of the mitochondrial network per se is not a required step in MOMP, fragmented mitochondrial networks may have a lowered threshold for MOMP as a result of altered architectural properties of mitochondria that permit more efficient Bax/Bak-dependent oligomerization within mitochondrial membranes. As further evidence of this, ablation of *Opa1*, which results in highly fragmented mitochondrial networks, results in either spontaneous apoptosis or increased susceptibility to a variety of proapoptotic triggers (Arnoult et al., 2005; Lee et al., 2004).

### Evidence for Direct Interactions between Bcl-2 Proteins and the Fission/Fusion Machinery

So, if members of the Bcl-2 family contribute to the regulation of mitochondrial dynamics, how does this operate at a molecular level? This is where confusion currently reigns, because interactions have been reported between several Bcl-2 family proteins, both pro- and antiapoptotic, and several core constituents of the mitochondrial fission/fusion machinery. Bak has been reported to interact with Mfn1 and Mfn2 (Brooks et al., 2007). Bcl-xL and Bcl-2 have been reported to interact with Mfn2, but not Mfn1 (Delivani et al., 2006), and Drp1 has been found to interact with and to exhibit enhanced activity in response to Bcl-xL (Berman et al., 2009). Furthermore, worm CED-9 has recently been found to interact with Fzo-1, the worm homolog of Mfn1/Mfn2 (Rolland et al., 2009). As yet, no consensus has emerged as to how Bcl-2 family members regulate mitochondrial fission and fusion dynamics, but it is plausible that members of this family could influence mitochondrial fission through stimulating Drp1 activity, as has been proposed (Berman et al., 2009), or through inhibiting interactions between Mfn1 and/or Mfn2. Similarly, because some members of the family have also been reported to promote mitochondrial fusion, this could occur as a result of enhancing Mfn1/Mfn2 interactions (Delivani et al., 2006; Rolland et al., 2009) or inhibiting Drp-1 function. Further studies are required to explore the range of interactions that members of the Bcl-2 family and mitochondrial morphogenesis proteins engage in, and to clarify the functional impact of these interactions.

### Conclusion and Perspectives

As outlined above, evidence now suggests that members of the Bcl-2 family can influence the dynamics of mitochondrial fission and fusion. Precisely how this occurs remains unclear, but is likely to be mediated via direct interactions between one or more members of the Bcl-2 family and core components of the mitochondrial fission/fusion machinery, such as Drp1 and Mfn2. At present, it seems highly unlikely that Bcl-2 family proteins act as key constituents of the mitochondrial fission/fusion machinery but rather as regulatory elements of this machinery, in a manner analogous to the way in which antiapoptotic Bcl-2 family proteins can regulate the autophagy machinery by interacting with Beclin-1 (Pattingre et al., 2005). Because Bcl-2 family proteins play such prominent roles as regulators of cell survival, it is perhaps unsurprising that members of this family may also survey the integrity of the mitochondrial network, given the importance of mitochondrial networks in cellular homeostasis.

It is worth noting that, apart from their central role in regulating cell death, Bcl-2 family proteins have also been implicated in seemingly diverse processes such as cell-cycle progression (Zinkel et al., 2006), inflammatory cytokine production (Bruey et al., 2007), and glucose metabolism (Danial et al., 2008) in recent years. Moreover, in *Drosophila*, where two Bcl-2 family members have been found, neither appears to be centrally involved in regulating MOMP or apoptosis in this organism. This begs the question as to what role, if any, these proteins subserve in the regulation of apoptosis in the fly. One possibility is that the Bcl-2 family has become an evolutionary remnant in

*Drosophila*. Another is that these proteins have been retained for the purpose of regulating mitochondrial morphogenesis in response to stress. Indeed, apoptosis-associated mitochondrial fragmentation has been observed in the fly (Goyal et al., 2007), despite the absence of a clear role for mitochondrial constituents for apoptosis in this organism. Thus, it remains unclear why mitochondrial fragmentation occurs during apoptosis in *Drosophila*, but this may again reflect apoptosis-associated perturbation of Bcl-2 family proteins (and, in turn, mitochondrial networks) unrelated to MOMP.

In a similar way, although CED-9 has also been found to be capable of influencing mitochondrial fission-fusion dynamics in mammalian cells and in the worm (Delivani et al., 2006; Li et al., 2008; Rolland et al., 2009), mitochondrial-derived factors do not appear to play any role in programmed cell death in this organism. Again, this leads us to ponder why CED-9 can impact upon mitochondrial network dynamics in the apparent absence of a role for mitochondria in worm cell death.

In conclusion, in addition to their role as regulators of MOMP within the cell death machinery, members of the Bcl-2 family may extend their sphere of influence by impacting upon processes—such as mitochondrial fission/fusion dynamics—that have long-range effects on cell viability, well before a cell is challenged with a direct proapoptotic insult.

### ACKNOWLEDGMENTS

We thank Science Foundation Ireland (SRCG20336 and 08/IN.1/B203) for support of work in the Martin laboratory.

### REFERENCES

- Arnout, D., Grodet, A., Lee, Y.J., Estaquier, J., and Blackstone, C. (2005). 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.
- Benard, G., Bellance, N., James, D., Parrone, P., Fernandez, H., Letellier, T., and Rostagnol, R. (2007). Mitochondrial bioenergetics and structural network organization. *J. Cell Sci.* *120*, 838–848.
- Berman, S.B., Chen, Y.B., Qi, B., McCaffery, J.M., Rucker, E.B., 3rd, Goebels, S., Nave, K.A., Arnold, B.A., Jonas, E.A., Pineda, F.J., and Hardwick, J.M. (2009). Bcl-xL increases mitochondrial fission, fusion, and biomass in neurons. *J. Cell Biol.* *184*, 707–719.
- 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. USA* *104*, 11649–11654.
- Bruey, J.M., Bruey-Sedano, N., Luciano, F., Zhai, D., Balpai, R., Xu, C., Kress, C.L., Bailly-Maitre, B., Li, X., Osterman, A., et al. (2007). Bcl-2 and Bcl-XL regulate proinflammatory caspase-1 activation by interaction with NALP1. *Cell* *129*, 45–56.
- Cassidy-Stone, A., Chipuk, J.E., Ingberman, 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. *Dev. Cell* *14*, 193–204.
- Chan, D.C. (2006). Mitochondria: dynamic organelles in disease, aging, and development. *Cell* *125*, 1241–1252.
- Chen, H., and Chan, D.C. (2005). Emerging functions of mammalian mitochondrial fusion and fission. *Hum. Mol. Genet.* *14 Spec No. 2*, R283–R289.

- 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.
- 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, C.J., Kono, H., Golenbock, D., Reed, G., Akira, S., and Rock, K.L. (2007). Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat. Med.* 13, 851–856.
- Claude, A., and Fullam, E.F. (1945). An electron microscope study of isolated mitochondria. *J. Exp. Med.* 87, 51–65.
- 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., et al. (2007). GAPDH and autophagy preserve survival after apoptotic cytochrome c release in the absence of caspase activation. *Cell* 129, 983–997.
- Danial, N.N., Walensky, L.D., Zhang, C.Y., Choi, C.S., Fisher, J.K., Molina, A.J., Datta, S.R., Pitter, K.L., Bird, G.H., Wikstrom, J.D., et al. (2008). Dual role of proapoptotic BAD in insulin secretion and beta cell survival. *Nat. Med.* 14, 144–153.
- 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. *Mol. Cell* 21, 761–773.
- Desagher, S., and Martinou, J.C. (2000). Mitochondria as the central control point of apoptosis. *Trends Cell Biol.* 10, 369–377.
- 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. *Mol. Cell* 30, 369–380.
- 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.
- 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.
- 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. *Dev. Cell* 1, 515–525.
- 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.
- Goyal, G., Fell, B., Sarin, A., Youle, R.J., and Sriram, V. (2007). Role of mitochondrial remodeling in programmed cell death in *Drosophila melanogaster*. *Dev. Cell* 12, 807–816.
- Griparic, L., van der Wel, N.N., Orozco, I.J., Peters, P.J., and van der Bliek, A.M. (2004). Loss of the intermembrane space protein Mgm1/OPA1 induces swelling and localized constrictions along the lengths of mitochondria. *J. Biol. Chem.* 279, 18792–18798.
- Ishihara, N., Nomura, M., Jofuku, A., Kato, H., Suzuki, S.O., Masuda, K., Otera, H., Nakanishi, Y., Nonaka, I., Goto, Y.I., et al. (2009). Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. *Nat. Cell Biol.* 11, 958–966.
- 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., 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.
- Karbowski, M., Arnoult, D., Chen, H., Chan, D.C., Smith, C.L., and Youle, R.J. (2004). 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., 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.
- Kono, H., and Rock, K.L. (2008). How dying cells alert the immune system to danger. *Nat. Rev. Immunol.* 8, 279–289.
- 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.
- Kowaltowski, A.J., Cosso, R.G., Campos, C.B., and Fiskum, G. (2002). Effect of Bcl-2 overexpression on mitochondrial structure and function. *J. Biol. Chem.* 277, 42802–42807.
- Kroemer, G., and Martin, S.J. (2005). Caspase-independent cell death. *Nat. Med.* 11, 725–730.
- 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. *Mol. Cell* 17, 525–535.
- 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.
- Legros, F., Lombes, A., Frachon, P., and Rojo, M. (2002). Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins. *Mol. Biol. Cell* 13, 4343–4354.
- Li, Z., Okamoto, K., Hayashi, Y., and Sheng, M. (2004). The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell* 119, 873–887.
- Li, H., Chen, Y., Jones, A.F., Sanger, R.H., Collis, L.P., Flannery, R., McNay, E.C., Yu, T., Schwarzenbacher, R., Bossy, B., et al. (2008). Bcl-xL induces Drp1-dependent synapse formation in cultured hippocampal neurons. *Proc. Natl. Acad. Sci. USA* 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 Genet.* 4, e1000097. 10.1371/journal.pgen.1000097.
- Luthi, A.U., and Martin, S.J. (2007). The CASBAH: a searchable database of caspase substrates. *Cell Death Differ.* 14, 641–650.
- Mitra, K., Wunder, C., Roysam, B., Lin, G., and Lippincott-Schwartz, J. (2009). A hyperfused mitochondrial state achieved at G1-S regulates cyclin E buildup and entry into S phase. *Proc. Natl. Acad. Sci. USA* 106, 11960–11965.
- Mootha, V.K., Wei, M.C., Buttle, K.F., Scorrano, L., Panoutsakopoulou, V., Mannella, C.A., and Korsmeyer, S.J. (2001). A reversible component of mitochondrial respiratory dysfunction in apoptosis can be rescued by exogenous cytochrome c. *EMBO J.* 20, 661–671.
- Mozdy, A.D., McCaffery, J.M., and Shaw, J.M. (2000). Dnm1p GTPase-mediated mitochondrial fission is a multi-step process requiring the novel integral membrane component Fis1p. *J. Cell Biol.* 151, 367–380.
- Munoz-Pinedo, C., Guio-Carrion, A., Goldstein, J.C., Fitzgerald, P., Newmeyer, D.D., and Green, D.R. (2006). Different mitochondrial intermembrane space proteins are released during apoptosis in a manner that is coordinately initiated but can vary in duration. *Proc. Natl. Acad. Sci. USA* 103, 11573–11578.
- 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.
- Parone, P.A., Da Cruz, S., Tondera, D., Mattenberger, Y., James, D.I., Maechler, P., Barja, F., and Martinou, J.C. (2008). Preventing mitochondrial fission impairs mitochondrial function and leads to loss of mitochondrial DNA. *PLoS ONE* 3, e3257. 10.1371/journal.pone.0003257.



- Pattingre, S., Tassa, A., Qu, X., Garuti, R., Liang, X.H., Mizushima, N., Packer, M., Schneider, M.D., and Levine, B. (2005). Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* 122, 927–939.
- Poncet, D., Pauleau, A.L., Szabadkai, G., Voza, A., Scholz, S.R., Le Bras, M., Brière, J.J., Jallil, A., Le Moigne, R., Brenner, C., et al. (2006). Cytopathic effects of the cytomegalovirus-encoded apoptosis inhibitory protein vMIA. *J. Cell Biol.* 174, 985–996.
- Puthalakath, H., and Strasser, A. (2002). Keeping killers on a tight leash: transcriptional and post-translational control of the pro-apoptotic activity of BH3-only proteins. *Cell Death Differ.* 9, 505–512.
- 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.
- Roumier, T., Szabadkai, G., Simoni, A.M., Perfettini, J.L., Paulau, A.L., Castedo, M., Metivier, D., Badley, A., Rizzuto, R., and Kroemer, G. (2006). HIV-1 protease inhibitors and cytomegalovirus vMIA induce mitochondrial fragmentation without triggering apoptosis. *Cell Death Differ.* 13, 348–351.
- Scorrano, L., Ashiya, M., Buttle, K., Weiler, S., Oakes, S.A., Mannella, C.A., and Korsmeyer, S.J. (2002). A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome *c* during apoptosis. *Dev. Cell* 2, 55–67.
- 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. *Mol. Cell* 31, 570–585.
- Suen, D.F., Norris, K.L., and Youle, R.J. (2008). Mitochondrial dynamics and apoptosis. *Genes Dev.* 22, 1577–1590.
- 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 Ca<sup>2+</sup> waves and protects against Ca<sup>2+</sup>-mediated apoptosis. *Mol. Cell* 16, 59–68.
- 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. *Nat. Rev. Mol. Cell Biol.* 9, 231–241.
- Tondera, D., Grandemange, S., Jourdain, A., Karbowski, M., Mattenberger, Y., Herzig, S., Da Cruz, S., Clerc, P., Raschke, I., Merkwirth, C., et al. (2009). SLP-2 is required for stress-induced mitochondrial hyperfusion. *EMBO J.* 28, 1589–1600.
- 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.
- Willis, S.N., Fletcher, J.I., Kaufmann, T., van Delft, M.F., Chen, L., Czabotar, P.E., Ierino, H., Lee, E.F., Fairlie, W.D., Bouillet, P., et al. (2007). Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. *Science* 315, 856–859.
- Wolter, K.G., Hsu, Y.T., Smith, C.L., Nechushtan, A., Xi, X.G., and Youle, R.J. (1997). Movement of Bax from the cytosol to mitochondria during apoptosis. *J. Cell Biol.* 139, 1281–1292.
- Youle, R.J., and Karbowski, M. (2005). Mitochondrial fission in apoptosis. *Nat. Rev. Mol. Cell Biol.* 6, 657–663.
- Youle, R.J., and Strasser, A. (2008). The BCL-2 protein family: opposing activities that mediate cell death. *Nat. Rev. Mol. Cell Biol.* 9, 47–59.
- Zinkel, S., Gross, A., and Yang, E. (2006). BCL-2 family in DNA damage and cell cycle control. *Cell Death Differ.* 13, 1351–1359.