Chapter 8. The mitochondrial pathways of apoptosis

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Abstract

Apoptosis is a process of programmed cell death that serves as a major mechanism for the precise regulation of cell numbers, and as a defense mechanism to remove unwanted and potentially dangerous cells. Studies in nematode, *Drosophila* and mammals have shown that, although regulation of the cell death machinery is somehow different from one species to another, it is controlled by homologous proteins and involves mitochondria. In mammals, activation of caspases (cysteine proteases that are the main executioners of apoptosis) is under the tight control of the Bcl-2 family proteins, named in reference to the first discovered mammalian cell death regulator. These proteins mainly act by regulating the release of caspases activators from mitochondria. Although for a long time the absence of mitochondrial changes was considered as a hallmark of apoptosis, mitochondria appear today as the central executioner of apoptosis. In this chapter, we present the current view on the mitochondrial pathway of apoptosis with a particular attention to new aspects of the regulation of the Bcl-2 proteins family control of mitochondrial membrane permeabilization: the mechanisms implicated in their mitochondrial targeting and activation during apoptosis, the function(s) of the oncosuppressive protein p53 at the mitochondria and the role of the processes of mitochondrial fusion and fission.

8.1. Introduction

Soon after it was recognized that organisms are made of cells, cell death was discovered as an important part of life. First observed during amphibian metamorphosis, normal cell death was soon found to occur in many developing tissues in both invertebrates and vertebrates (reviews: (Clarke, 1990, Clarke and Clarke, 1996)). The term "programmed cell death" (PCD) was used to describe cell deaths that occur in predictable places and at predictable times during development, to emphasize that death can be somewhat programmed into the development plan of the organism. Subsequently it has been established that PCD also serves as a major mechanism for the precise regulation of cell numbers and as a defense mechanism to remove unwanted and potentially dangerous cells, such as self-reactive lymphocytes, cells that have been infected by viruses and tumor cells. In addition to the beneficial effects of PCD, the inappropriate activation of cell death may cause or contribute to a variety of diseases, including acquired immunodeficiency syndrome (AIDS), neurodegenerative diseases, and ischemic strokes. Conversely, a defect in PCD activation could be responsible for some autoimmune diseases and is also involved in oncogenesis.

Apoptosis is a process whereby cells activate an intrinsic cell suicide program that is one of the potential cellular responses, such as differentiation and proliferation. It has been defined in 1972 by Kerr *et al.* in contrast to necrosis, which is a cell death generally due to aggressions from the external medium (Kerr et al., 1972). The apoptotic process is associated with characteristic morphological and biochemical changes, such as membrane blebbing, cell shrinkage, chromatin condensation, DNA cleavage and fragmentation of the cell into membrane-bound apoptotic bodies whose surface expresses potent triggers for phagocytosis. However, it must be kept in mind that although apoptosis is the most common form of PCD, dying cells may follow other morphological types (Bredesen et al., 2006). This chapter reviews our current knowledge on the mitochondrial pathway of apoptosis with a particular emphasis to new aspects of the regulation of the Bcl-2 proteins family control of mitochondrial membrane permeabilization (MOMP) and the mechanisms implicated in their mitochondrial targeting and activation, the function(s) of the oncosuppressive protein p53 at the mitochondria and the role of the processes of mitochondrial fusion and fission.

8.2 The various roles of mitochondria in apoptosis

8.2.1. Insights from studies in invertebrate models

Genetic screens performed in *C. elegans* have allowed the discovery of a genetic control of PCD and elucidation of the signaling cascade leading to cell death (for review see (Lettre and Hengartner, 2006)). At the heart of this pathway is the *ced-3* gene, which encodes a member of a family of cysteine proteases that cleave proteins at specific aspartyl residues called caspases, whose activation is under the control of the caspase activator CED-4. In living cells, CED-4 is constantly

sequestered by CED-9 on the mitochondrial outer membrane. Upon a proapoptotic stimuli, EGL-1 is transcriptionally activated and binds to CED-9 to displace CED-4 (figure 1). CED-4 and CED-3 can then interact through their CARD (caspase recruitment domain) domains to form a complex called apoptosome that leads to CED-3 activation (figure 1). Apoptosome formation and activation constitute key events for cell death execution in worm in a step controlled by EGL-1 and CED-9. Activated CED-3 will then cleave cellular components leading to cell destruction and engulfing. Subsequent studies, performed in various species, have shown that caspases are also instrumental to the execution of apoptosis in other species. These enzymes are expressed in cells as inactive or low-activity zymogens that require oligomerization and/or cleavage for activation. In *C. elegans*, CED-3 is able to autocatalyze its own cleavage (Hugunin et al., 1996). As CED-3 is the only apoptotic caspase in the worm, it plays a central role in apoptosis execution in this organism (Ellis and Horvitz, 1986, Shaham et al., 1999).

Studies performed in *Drosophila* with p35 (a baculovirus caspases inhibitor) have shown that caspases are also involved in apoptosis in fruit flies. However, regulation of caspase activation in flies appears to be mainly controlled at another level. A genetic screen of a deletion mutant library showed that the H99 deletion abolishes almost all cell death during embryogenesis in *Drosophila* (White et al., 1994). This phenotype is the consequence of the loss of three genes: *Reaper*, *Hid*, and *Grim*, collectively called RHG proteins. In healthy cells, IAPs, caspase inhibitor proteins that contain a RING domain and Baculoviral IAP repeat (BIR) motifs such as DIAP1 cause the ubiquitylation of procaspases, thereby inactivating them (for a recent review, see (Bergmann, 2010)). In response to an apoptotic stimulus, RHG proteins are activated, leading to the RHG protein-dependent ubiquitylation and proteosomal degradation of DIAP1 (Chai et al., 2003, Goyal et al., 2000, Wilson et al., 2002, Yoo et al., 2002). Overexpression of any one of the RHG genes triggers excessive cell death, indicating that the inhibition of IAP is sufficient to induce caspase activation and apoptosis. Consistently, DIAP1 deficiency leads to spontaneous apoptosis in most fly cells (Goyal et al., 2000, Yoo et al., 2002). These data led to the concept that *Drosophila* caspases might not require activation, but simply relief from potent inhibitors of caspases. However, the *Drosophila* initiator caspase DRONC contains in its aminoterminal region a long prodomain that carries a CARD motif which mediates DRONC binding to Dark/Dapaf-1, the *Drosophila* homologue of the nematode CED-4 caspase activator, and the formation of the fly apoptosome (Daish et al., 2004, Xu et al., 2005). Furthermore, analysis of the *Drosophila* genome allowed the discovery of two homologues of CED-9 (*i.e.* Debcl and Buffy), which are constitutively located at the mitochondria (Figure 1). The first one is a proapoptotic member called Debcl (Colussi et al., 2000, Brachmann et al., 2000, Igaki et al., 2000), and the second is the antiapoptotic Buffy (Quinn et al., 2003). These data show that regulation of caspases activation is somehow conserved between worms and *Drosophila* and suggest that critical events take place at the mitochondrial level.

8.2.2. The mitochondrial pathway of apoptosis in mammals

In mammals, apoptosis induction also usually leads to caspase activation even though not all of caspase activities are linked to cell death commitment and apoptosis can proceed in some instances without caspase activity (Godefroy et al., 2004). Caspases can be classified into two subgroups: the first one is constituted by effector caspases which present a short prodomain and whose activating cleavage is performed by other proteases (such as caspases or calpain). The second group is constituted by initiator or apical caspases, which present a long prodomain carrying a protein/protein interaction motif dubbed ”death domain” (Park et al., 2007). This motif can either be a CARD (Caspase Activation and Recruitment Domain) or a DED (Death Effector Domain) domain. Initiator caspases are characterized by their ability to autoactivate within specialized complexes (Bao and Shi, 2007, Ho and Hawkins, 2005, Stennicke and Salvesen, 2000). Two main pathways lead to caspase activation during apoptosis in mammals. The first one involves transmembrane receptors at the plasma membrane and is thus termed extrinsic pathway or death-receptor pathway (for review: (Guicciardi and Gores, 2009)). The second one, which is more similar to the worm death pathway, is termed intrinsic or mitochondrial pathway and places mitochondria at the core of the signaling cascade (for review: (Wang and Youle, 2009)).

However, for a long time the absence of mitochondrial changes was considered as a hallmark of PCD (Kerr et al., 1972, Kerr and Harmon, 1991) and it was thus postulated that apoptosis is controlled at the nuclear level. This theory was however challenged during the 90's by several lines of evidence. First, the Bcl-2 protooncogene, responsible for B cell follicular lymphomas due to t(14;18) chromosomal translocations, and other Bcl-2 related proteins like Bcl-x_L, were found to be negative regulators of cell death, able to prevent cells from undergoing apoptosis induced by various stimuli in a wide variety of cell types (Korsmeyer, 1992, Zhong et al., 1993). Although, the mechanism(s) by which proteins of the Bcl-2 family modulate apoptosis was not known, it was observed that most members of the Bcl-2 family proteins are localized to the nuclear envelope, the endoplasmic reticulum and the outer mitochondrial membrane. Furthermore, this membrane association seemed of functional significance, as mutant Bcl-2 molecules lacking this membrane anchorage capacity were found less effective at preventing apoptosis in some systems (Borner et al., 1994, Nguyen et al., 1994, Zhu et al., 1996). Second, several changes in mitochondrial biogenesis and function were found associated with the commitment to apoptosis. A fall of the membrane potential ($\Delta\Psi m$) occurs before the fragmentation of the DNA in oligonucleosomal fragments (Vayssiere et al., 1994, Petit et al., 1995, Zamzami et al., 1995). This drop of $\Delta\Psi m$ is responsible for changes in mitochondrial biogenesis and activity (Vayssiere et al., 1994). These data showed that the nuclear fragmentation is a late event as compared to mitochondrial changes. Last, translocation of cytochrome c from mitochondria to cytosol has been shown to be a crucial step in the activation of the apoptosis machinery in various cell death models and in a cell-free system using *Xenopus* egg extracts or dATP-primed cytosol of growing cells

(Liu et al., 1996, Kluck et al., 1997b). Once released, cytochrome c, in interaction with the apoptosis protease-activating factor 1 (Apaf-1), triggers the initiator caspases-9 activation, which leads to the subsequent characteristic features of apoptosis, including chromatin condensation and nuclear fragmentation, cleavage of fodrin, PARP and Lamin B_1. Remarkably, part of the sequence of Apaf-1 shows a striking similarity to that of CED-4, with the two proteins aligning over most of the CED-4 sequence. Finally, a decisive observation was that release of cytochrome c is blocked by overexpression of Bcl-2 (Kluck et al., 1997a, Yang et al., 1997). These data firmly established an active role of mitochondrial outer membrane permeabilization (MOMP) in apoptosis. Soon after this discovery, mitochondria was found to release many other proteins that could participate in apoptosis. Smac/DIABLO was identified simultaneously by its ability to enhance cytochrome c-mediated caspase-3 activation (Du et al., 2000) and by its interaction with XIAP (Verhagen et al., 2000). It facilitates caspase activation by binding to IAPs, and removing their inhibitory activity in a way similar to that of *Drosophila* RHG proteins. Soon after, HtrA2/Omi was found to be another XIAP-binding protein (Hegde et al., 2002, Martins et al., 2002, Suzuki et al., 2001) released from mitochondria during apoptosis and exhibiting proapoptotic activity (van Loo et al., 2002). These data showed that, although regulation of caspase activation within the apoptosome is different to some extent between worm, *Drosophila* and mammals, it is controlled by homologous proteins (table 1) and involves mitochondria (for review: (Colin et al., 2009b, Wang and Youle, 2009)). Moreover, apart from this pivotal role of mitochondria in the control of caspases activation, it should be noticed that reactive oxygen species (ROS) produced by the mitochondria (see chapter 5) can be involved in apoptosis signaling (for reviewed: (Fleury et al., 2002)) and that Bcl-2 has been shown to regulate mitochondrial respiration and the level of different ROS (for review : (Chen and Pervaiz, 2009a)), at least in part through a control of cytochrome c oxidase activity (Chen and Pervaiz, 2009b).

8.2.3. Evolution of the mitochondrial pathway of apoptosis

In mammals, since apoptosome activation requires the release of cytochrome c from the mitochondrial intermembrane space to the cytosol, it is therefore subjected to the regulation of cytochrome c location. Two major models that are not mutually exclusive have been proposed to explain the Bcl-2 family proteins control of the MOMP (for review (Desagher and Martinou, 2000, Kroemer et al., 2007)). The first model involves a regulation of the opening of the PTP (Permeability Transition Pore), a macromolecular channel which includes ANT, VDAC, cyclophilin D and other variable components, Opening of PTP leads to matrix swelling, decrease of $\Delta\Psi m$, subsequent rupture of the outer membrane, and nonselective release of proteins located in the intermembrane space. *In vitro* experiments as well as experiments on isolated mitochondria indicate that both antiapoptotic and proapoptotic Bcl-2 family proteins could regulate the PTP opening. However, genetic analysis of mice KO for VDAC or ANT suggests that in most cases PTP opening might rather be a consequence of apoptosis. The second model relies on the formation in the mitochondrial outer membrane of channels

formed by some proapoptotic members of the Bcl-2 family allowing the release of proteins of the intermembrane space into the cytosol. In this model other members of the family can either activate or inhibit the formation of these channels. Recently, as reviewed in paragraph 8.5, a third model, involving proteins involved in the regulation of mitochondrial shape and dynamics, has also been proposed (see also: (Jourdain and Martinou, 2009, Wasilewski and Scorrano, 2009, Autret and Martin, 2009)). However, whatever the model, antiapoptotic members of the Bcl-2 family impair release of apoptogenic factors in the cytosol while proapoptotic ones favor this relocation.

In contrast to the mammalian apoptosome, the *Drosophila* apoptosome activation, although supposed to occur at or nearby mitochondria, has been shown to be mainly regulated by modulating its inhibition by IAP proteins. In fact, apoptosis induction leads to the release of the apoptosome from DIAP1 mediated inhibition (Muro et al., 2002). In this process of apoptosome activation, cytochrome c is clearly not crucial. In addition, although mammalian Bcl-2 inhibits apoptosis induced by various stimuli in *Drosophila* (Gaumer et al., 2000, Brun et al., 2002), *Drosophila* Bcl-2 family members do not seem to be key regulators of developmental apoptosis. Indeed, Debcl has a limited role in developmental apoptosis (Galindo et al., 2009) but could be important for stress-induced cell death (Sevrioukov et al., 2007). Moreover, the way this protein family regulates the *Drosophila* apoptosome is still unclear. Nevertheless, it seems that the apoptotic cascade is somehow inverted between flies and worm/mammals. Indeed, contrarily to what happens in these two organisms, in which apoptosis regulators are relocated from mitochondria to the cytosol, it seems that *Drosophila* apoptosis regulators use an opposite relocation to concentrate at or around mitochondria during apoptosis. Indeed, targeting of the RHG proteins Reaper and Grim to mitochondria involving a GH3 (Grim Homology 3) domain seems to be required for their proapoptotic activity (Claveria et al., 2002, Olson et al., 2003, Freel et al., 2008). Furthermore Hid possess a mitochondrial targeting sequence and Rpr requires Hid for recruitment to the mitochondrial membrane and for efficient induction of cell death in vivo (Sandu et al., 2010). In the nematode, it seems that a third mode of apoptosome control has been selected. Once more, Bcl-2 family members are important for regulation of apoptosome activity but this regulation, although involving CED-4 release from mitochondria-bound CED-9, occurs directly without involving MOMP as a decisive step. Taken together, studies of apoptosome activation in these different species show that the way Bcl-2 family proteins bound to mitochondria regulate caspase activity has evolved during evolution.

8.3 The Bcl-2 family

The discovery that Bcl-2 was the functional homolog of *C. elegans* cell survival protein CED-9 (Hengartner and Horvitz, 1994) and could prevent apoptosis in many systems led to the discoveries of homologous proteins exhibiting structures similar to Bcl-2 albeit not identical functions. Members of this family of proteins (hence dubbed the Bcl-2 family protein) share homologies restricted to 1 to 4

domains. Proteins with 4 Bcl-2 Homology domains (BH1-4) are anti apoptotic (*i.e.* Bcl-2, Bcl-X_L, Mcl-1…), other members of this family are proapoptotic (Chipuk et al.). Proapoptotic proteins are divided between multidomain proapoptotic proteins (Bax, Bak…), which exhibit a BH1-3 domains homology and proteins with homology essentially restricted to the BH3 domain and thus called the BH3 only proteins (BOPs) (Chipuk et al.). Apoptosis is tightly controlled, at early stages, by the interaction between members of the Bcl-2 family. If it has been clearly established that proteins such as Bax and Bak are responsible for the mitochondrial apoptotic permeability and that survival proteins like Bcl-2 are inhibitors of the latter processes, the role of BOPs is still a matter of a vivid debate (Chipuk et al., Giam et al., 2008). BOPs have been shown to fall into 2 categories, a group of proteins, which interact with all multidomain antiapoptotic proteins (*i.e.* BID, BIM and PUMA), while a second group exhibits distinct affinities toward antiapoptotic proteins of the family (*i.e.* BIK, BMF, BAD, NOXA, HRK…) (figure 2). Several models depicting the roles of the members of the Bcl-2 family in the control of Bax/ Bak activation have been proposed but after a decade of exciting but somehow contradictory results, the remaining questions point to the existence of a direct or indirect activation of proapoptotic Bax and Bak by BOPs (figure 3). Two theories are still discussed: one postulates that BOPs directly activate Bax or Bak and induce their change of conformation and mitochondrial integration by a “kiss and run” mechanism (Youle and Strasser, 2008). This has been shown for at least BID and BIM (Chipuk et al.), and appears to be also the case for PUMA (Cartron et al., 2004, Kim et al., 2009). Other BOPs act as mere inhibitors of antiapoptotic proteins (a property also shared by activators of Bax / Bak) (figure 2). It is not surprising that activators of Bax and Bak can also bind all the antiapoptotic members of the Bcl-2 family (cf figures 1 and 2). Indeed, the activity of this class of BOPs might require both features (Merino et al., 2009). The specificity of other BOPs for subclasses of survival proteins of the Bcl-2 family are supposed to be link to the specificity of these proteins to intervene in apoptosis. These BOPs are the most upstream apoptotic sensors because of their early and selective activation by tissue restricted and / or stress signals. For example, it has been shown that BAD was especially responsive to growth factors withdrawal and /or the impairment of glucose metabolism (Danial, 2008). Similarly, proteins like PUMA and NOXA are sensors of DNA damage in a p53 manner while BID is specifically activated by the Fas pathway (Youle and Strasser, 2008). However, in the past years, it has become evident that these proteins could also be involved in other cellular functions as diverse as cell cycle regulation, DNA repair and metabolism. For example, BAD resides in and controls the formation of a glucokinase complex that regulates mitochondria respiration (Danial et al., 2003). BAD is active in this complex as a phosphorylated protein and a glucose deficit triggers dephosphorylation of BAD and disassembly of the complex, which thus in turn amplifies BAD-induced death signal (Danial et al., 2003). This function appears to be playing an important role in the insulin secretion in beta cells, favoring physiological adaption of these cells to high fat diet (Danial et al., 2008). Proapoptotic BOP activity has been particularly studied in the case of BID, which upon cleavage by caspase 8 becomes tBID, a very efficient direct activator of BAX and

BAK (Luo et al., 1998, Li et al., 1998). As such, tBID conveys the apoptotic signal form cell surface death receptors to mitochondria (Youle and Strasser, 2008). However, it should be noted that BID, under its native form, has been involved in "life" functions and more particularly in cell cycle progression into S phase (Yin, 2006, Gross, 2006). It is thus likely that other functions of BOPs, especially mitochondrial ones, will be revealed in the future. One might suggest that members of the Bcl-2 family have evolved and adapted from specific functions to the control of apoptosis.

Mitochondria have been considered to be passive actors during apoptosis beyond the activation of PTP. However, in the past years, it has become obvious that this organelle could play an active role in its BAX/BAK-mediated permeabilization. Kuwana *et al*., (Kuwana et al., 2002) using a simple liposome system, have suggested that mitochondrial proteins are dispensable for BID-mediated insertion of Bax, as long as cardiolipin, a lipid mainly located in the inner membrane of the mitochondria, was added in important proportion. Cardiolopin is important for the insertion of BID in liposomes or mitochondria in conjunction with mitochondrial carrier homolog 2, a mitochondrial inner membrane transporter (Grinberg et al., 2005, Schug and Gottlieb, 2009). In that respect, it is important to note that this cardiolipin requirement was not found with BIM or oligomerized BAX (Schafer et al., 2009), suggesting that other agents such as proteins are needed in this process as discussed in Petit *et al.* (Petit et al., 2009). Indeed, several groups have postulated the existence of mitochondrial proteins involved in the docking and/or the insertion of Bax into mitochondria (reviewed in (Petit et al., 2009)).

The majority of mitochondrial proteins is encoded in the nucleus and thus is imported into mitochondria *via* different translocation complexes such as the translocase of the outer membrane (called the TOM complex) and the translocase of the inner membrane (called the TIM complex), as well as the sorting and the assembly machinery (the SAM complex) (Becker et al., 2009). The question of the involvement of the TOM complex in the docking of BAX during apoptosis has been raised by recent studies (Petit et al., 2009, Ott et al., 2009). This point is of importance as the signal of Bax addressing has not been clearly established yet (Petit et al., 2009). Nevertheless, several proteins of the Bcl-2 family have been shown to interact with proteins of the TOM complex namely Bcl-2 with TOM20 (Schleiff et al., 1997, Motz et al., 2002), Mcl-1 with TOM70 (Chou et al., 2006) and Bax with TOM22 (Bellot et al., 2007, Colin et al., 2009a) and TOM40 (Ott et al., 2007, Cartron et al., 2008). It should be noted that an unnatural C–terminal mutant of Bax (*i.e.* Bax Ser184Val) constitutively associates with mitochondria and does not interact with the TOM complex during TNF alpha-induced apoptosis (Ross et al., 2009). This result suggests that either the forced localization of Bax through mutations abrogated the requirement for TOM complex or that BAX interaction with TOM depends on the nature of the apoptotic signal. The exact implication of mitochondrial translocases during apoptosis remains thus to be established as well as its impact with mitochondrial physiology.

Contrary to BAX, which is a cytosolic protein in resting cells, BAK permanently resides at the mitochondrion. Cheng *et al.* (Cheng et al., 2003) have found that Bak complexes with a low abundant isoform of VDAC (VDAC2) in viable cells and that this association keeps BAK inactive. This step might provide a connection between the PTP and the Bcl-2 family members.

8.4. Functions of p53 on mitochondria

The p53 protein, first described about 30 years ago, was characterized as a tumor suppressor at the beginning of 1990 decade, and is nowadays the subject of almost 50 000 articles[1]. The p53 tumor suppressor protein plays a central role in the regulation of apoptosis, cell cycle and senescence as a response to a broad range of stresses such as DNA damage, oncogene activation and hypoxia; *p53* gene or its product was found to be inactivated in more than 50% of all human cancers. The crucial tumor suppressor activity of p53 involves both transcription-dependent and -independent mechanisms (Fridman and Lowe, 2003). Thus, p53 activates the transcription of genes that encode apoptotic effectors, such as PUMA, NOXA, BID, Bax, p53AIP1 proteins (Miyashita and Reed, 1995, Oda et al., 2000), and it represses the transcription of antiapoptotic genes such as *bcl-2* and *survivin* (Shen and Shenk, 1994, Hoffman et al., 2002). Beside these well-known activities, p53 has lately been described as regulating a wide spectrum of processes such as the metabolism (glycolysis, ROS homeostasis), autophagy, cell invasion and motility, angiogenesis, bone remodeling, etc (for review see (Vousden and Lane, 2007). For more than a decade now, many papers emerged describing the transcription-independent proapoptotic activities of p53, and its capacity to regulate the function of proteins involved in apoptosis commitment. Indeed, transactivation-incompetent p53 mutants can induce apoptosis in human cells as efficiently as wild type p53 (Caelles et al., 1994, Kakudo et al., 2005). Moreover, p53 can promote apoptosis when the nuclear import of p53 (and thereby its transcriptional activity) is inhibited (Chipuk et al., 2004). Transcription-independent pathways induced by p53 could play a primary role in gamma irradiation-induced apoptosis in mouse (Erster et al., 2004). Similarly, it was shown that upregulation of proapoptotic targets can be insufficient to induce apoptosis and requires further transcription-independent p53 signaling (Johnson et al., 2008).

The intensive study of these surprising pathways has identified mitochondria as a major site of transcriptional-independent apoptotic activity of p53. Indeed, numerous publications report that p53 itself relocates and induces apoptosis directly at mitochondria, *via* the interaction with members of the Bcl-2 family (Marchenko et al., 2000, Moll and Zaika, 2001). Moreover, some data suggest that p53 could also act in altering mitochondrial physiology to promote apoptosis. Last but not least, recent studies have revealed the importance of p53 under conditions of apparent normal growth and development, *i.e.* in the absence of chronic or severe stress (Vousden and Lane, 2007). For example,

[1] A search in PubMed database, with "p53" as query target, returns more than 52,000 results as of February 2010.

basal or low levels of p53 were shown to contribute to the maintenance and the activity of mitochondria (Bensaad and Vousden, 2007), in part through nuclear transcription-dependent mechanisms.

8.4.1 Mitochondrial localization of p53 in stress condition

A new paradigm for the transcription-independent apoptotic activities of p53 first emerged with the evidence of the possible accumulation of p53 in the cytosol or mitochondria in response to stress (Green and Kroemer, 2009). It was reinforced by the demonstration that p53 could activate the intrinsic apoptotic pathway by directly inducing mitochondrial outer membrane permeabilization (MOMP) and triggering the release of proapoptotic factors from the intermembrane space. Indeed, the accumulation of mitochondrial p53 was described in a variety of experimental systems - from transformed cell lines to animal models - and was shown to correlate with transcription-independent mechanisms. For example, irradiation of mice or ischemic damage of the brain promotes the translocation of p53 to the mitochondrion. Similarly, *in vitro* studies illustrated the ability of p53 to interact with isolated mitochondria and to promote MOMP.

Numerous data suggest that the transcription-independent activity of p53 at the mitochondrial level is dependent on a primary interaction with members of the Bcl-2 family that leads to the induction of MOMP; p53 acting somehow as a BH3 only protein. The direct or indirect activation of Bak and Bax proapoptotic members plays a central role in this mechanism. Binding of p53 to Bak, an intrinsic outer mitochondrial membrane, was found to catalyze Bak activation and cytochrome c release (Leu et al., 2004). Characterization of the p53–Bak interaction revealed the crucial importance of the DNA-binding domain of p53 for interacting and oligomerizing with Bak (Leu et al., 2004, Pietsch et al., 2008). However, some results indicate that binding of p53 to Bak is not sufficient to induce apoptosis *in vivo*. Indeed, overexpression in human cancer cell lines of a transcriptionally impaired p53 showed that although p53 was able to bind to Bak it did not exhibit apoptotic activity (Mihara et al., 2003, Pietsch et al., 2008). Cytosolic accumulation of p53 was also shown to promote transcription-independent activation of Bax *in vivo* (Tan et al., 2005, Speidel et al., 2006, Akhtar et al., 2006, Geng et al., 2007) according to a ‘hit-and-run’ model involving the proline-rich domain of p53 that is located near its N-terminus (Chipuk et al., 2004). In addition to the interactions with Bax and Bak, p53 was reported to bind with the antiapoptotic Bcl-2 family proteins Bcl-X_L and Bcl-2 through its DNA-binding domain (Petros et al., 2004, Tomita et al., 2006, Sot et al., 2007). Mutations within this domain abolish the binding of p53 to Bcl-xL/Bcl-2 (Mihara et al., 2003). Distinct models have been proposed to explain the function of the Bcl-X_L/Bcl-2–p53 interactions. Some data suggest that binding of p53 to these proteins counteracts their inhibitory action on proapoptotic members of the Bcl-2 family (Bak and Bax) (Mihara et al., 2003). In this way, p53 mutants that are defective in the ability to bind Bcl-X_L/Bcl-2 also lack apoptotic activity, which is in agreement with the fact that p53

abolishes the antiapoptotic function ability of Bcl-2 and Bcl-xL (Jiang et al., 2006, Fletcher et al., 2008). Conversely, other results point to a model in which complexes between p53 and antiapoptotic Bcl-2 proteins are rather a sequestering mechanism that inhibits apoptotic functions of p53. Indeed, apoptosis induction has correlated with disruption of the Bcl-X_L –p53 complex rather than an increase in its formation (Chipuk et al., 2005). Expression of a Bcl-X_L unable to bind proapoptotic Bcl-2 members inhibited p53-induced apoptosis suggesting that Bcl-X_L can inhibit apoptotic function of p53.

The importance of p53 activity at the mitochondrial level was underscored by the observed effect of pifithrin-μ, a drug that reduces the binding of p53 to mitochondria but has no apparent effect on p53-dependent transactivation (Strom et al., 2006). This drug probably blocks the interaction of p53 with anti- and proapoptotic Bcl-2 family members. Pifithrin-μ can protect thymocytes from otherwise lethal irradiation altrough the upregulation of p53 target genes was not affected. In the same way, this drug reduces apoptosis of human embryonic stem cells upon UV irradiation (Qin et al., 2007). Conversely, it was shown that the proapoptotic effect of a drug as CP-31398 correlated with the translocation of p53 to mitochondria and the induction of a p53-dependent MOMP (Tang et al., 2007).

Several studies have confirmed the *in vivo* and physiological relevance of transcription-independent mechanisms in p53-mediated apoptosis. It has been shown that upregulation of proapoptotic p53 target genes is not sufficient to induce apoptosis following irradiation of mouse fibroblasts. The transcription-independent signaling, *i.e.* the activation of Bax by cytosolic p53, provides the decisive signal for the onset of cell death (Speidel et al., 2006). Similarly, mouse embryo fibroblasts expressing a p53 variant that was transcriptionally active but unable to interact with Bax were resistant to genotoxic stress although proapoptotic target genes were strongly activated (Johnson et al., 2008). Furthermore, it was described that the onset of apoptosis correlated with the translocation of p53 to mitochondria in sensitive tissues after gamma irradiation of mice (Erster et al., 2004).

Besides the evidences of a direct proapoptotic action of p53 on mitochondria, some results add complexity to the significance of the mitochondrial location of p53 in stress condition. First, mitochondrial p53 has been detected not only in apoptotic conditions but also associated to a growth arrest response to drug treatment; however the connection between the two events is not yet well established (Mahyar-Roemer et al., 2004, Essmann et al., 2005). Second, most data indicate that p53 is located to, or near, the outer membrane where it interacts with proteins of the Bcl-2 family to promote MOMP and apoptosis, but some publications suggest that a part of p53 is found within the mitochondria, in the matrix or associated to the inner membrane. Localization of p53 into the mitochondrion could be correlated to a transcription-independent role of p53 in the maintenance and stability of the mitochondrial genome. Indeed, p53 was shown to directly interact with mtDNA polymerase γ and to consequently enhance the DNA replication function of polymerase γ (Achanta et

al., 2005). Moreover, p53 could bind directly to the mitochondrial base excision repair machinery (mtBER) (Chen et al., 2006) to remove damaged bases and stimulate repair mechanisms. Moreover, p53 is also able to bind the mitochondrial transcription factor A (mtTFA) in order to regulate both transcription and repair of mtDNA (Yoshida et al., 2003). Altogether, these data suggest that p53 can also exert a protective effect on mitochondria thanks to mechanisms that are independent of its nuclear transcriptional activities. In this model, p53 would reduce the risk of mtDNA mutations and mitochondrial malfunctions in the presence of ROS or DNA-damaging agents. This would explain both the increased genetic instability associated with the loss of p53 function in the late stage of cancer development and that mtDNA mutations promote aggressive tumor behavior (Petros et al., 2005, Shidara et al., 2005).

8.4.2 Mitochondrial localization of p53 in the absence of stress

If the localization of p53 in conditions of stress is now relatively well documented, there are few literature data concerning the localization of p53 in the absence of stress. In this condition, p53 is assumed to be maintained at a basal protein level *via* Mdm2-induced poly-ubiquitination and degradation (Haupt et al., 1995, Grossman et al., 2003). Some studies have shown that Mdm2-mediated mono-ubiquitination leads to the export of p53 from the nucleus to the cytoplasm (Li et al., 2002, Brooks and Gu, 2006). Once in the cytoplasm the major part of p53 is degraded by the proteasome. However, low levels of p53 are still present in normal tissues. According to the authors, in such conditions p53 is exclusively localized either in the nucleus or in the cytoplasm, depending on the cell status (normal or tumor/transformed cells). Some data suggest a cytoskeleton associated location of wild type p53 either with the actin filaments (Katsumoto et al., 1995), or with the microtubule network (Giannakakou et al., 2000). The interaction with microtubules is mediated by a motor protein (dynein), which in conditions of stress participates to the transport of p53 toward the nucleus using the microtubule network as a “highway”. In certain human cancers such as breast cancers, colon cancers and neuroblastoma, wild type p53 is only detected in the cytoplasm (Moll et al., 1995, Bosari et al., 1995). Shuttling the protein out of the nucleus is an efficient way to control the activity of a tumor suppressor protein that acts as a transcription factor. Cytoplasmic sequestration of wild type p53 in these cancers has been associated with their poor response to chemotherapy and radiation therapy. In this case, proteins such as Parc have been shown to be cytoplasmic anchors for wild type p53, that prevent p53 transport to the nucleus (Nikolaev et al., 2003). Nevertheless, cytoplasmic sequestration of p53 can also be associated to a physiological occurrence in some cell types such as in the mammary gland during lactation (Moll et al., 1992) or in embryonic stem cells (Aladjem et al., 1998) to permit transient proliferation.

Furthermore, some data indicate that p53 could be located at mitochondria in the absence of stress. A first report showed a direct positive influence of a mitochondria targeted p53 on

mitochondrial biogenesis and function (Donahue et al., 2001). We demonstrate that wild type p53 can be localized at mitochondria in living and proliferative cells issued from different species and regardless of the cell status (tumor, immortalized or primary cells) (Ferecatu et al., 2009). This mitochondrial localization of p53 in normal conditions agrees with recent observations of a direct positive influence of p53 on the biogenesis and the activity of mitochondria in part through nuclear transcription-dependent mechanisms (Bensaad and Vousden, 2007).

Besides a role in mitochondria biogenesis, we cannot rule out the possibility that mitochondrial p53 represents a pool of p53 at the outer membrane; which could induce outer membrane permeabilization through physical interaction with members of the Bcl-2 family members following induction of apoptosis. Organelle location of p53 may also represent a way to sequestrate the tumor suppressor under normal conditions.

8.4.3. Mitochondrial targeting of p53

p53 stabilization and activation depend on a series of post-translational modifications that include phosphorylation, acetylation, methylation, ubiquitination, sumoylation neddylation, glycosylation and rybosylation. Post-translational modifications also provide key signals for the cellular trafficking of p53 between organelles, although interaction with specific factors may also be involved in this process. Because p53 seems to be primarily a nuclear protein, its nuclear export and abundance in the cytosol could determine its ability to interact with mitochondria.

p53's ubiquitination is crucial in its nucleo-cytoplasmic shuttling, and is mediated by the Mdm2 protein. p53 is ubiquitinated by Mdm2 within the nucleus, unmasking the nuclear export signal (NES) of p53 and permitting p53 exit through the nuclear pores into the cytoplasm (Gottifredi and Prives, 2001, Geyer et al., 2000). Mdm2 alone only catalyzes monoubiquitination of p53 (at one or multiple sites) and p53 poly-ubiquitination, which addresses p53 to the proteasome, involves a cytosolic cofactor, p300, which mediates the formation of a complex containing both p53 and the proteasome (Grossman et al., 2003, Lai et al., 2001). Moreover, low levels of Mdm2 induce monoubiquitination and nuclear export of p53 whereas high levels of Mdm2 promote polyubiquitination and degradation by nuclear proteasomes (Li et al., 2002).

Acetylation regulates both the stability of p53 by inhibiting Mdm2-induced ubiquitination as they occur at the same sites (Li et al., 2002) and the sub-cellular localization of p53. It was shown that p53 hyperacetylation prevents p53 oligomerization and determines the cytoplasmic accumulation of p53 by exposing the NES (Kawaguchi et al., 2006). The acetylation of more than four lysines promotes p53 export to the cytoplasm but no functional role has yet been associated to such hyper-acetylation of p53.

Since the structure of p53 does not harbor a typical mitochondrial targeting sequence, the mechanisms responsible for p53 mitochondrial localization remain unclear. Only few studies concern signals that address p53 to mitochondria in conditions of stress and that govern its interaction with members of the Bcl-2 family members to promote MOMP. Post-translational modifications have been studied as putative mitochondria translocation signals. Primary data indicate that neither acetylation nor phosphorylation seems to be involved in p53 targeting to mitochondria (Mahyar-Roemer et al., 2004, Nemajerova et al., 2005). However, it was recently shown that Ser15 phosphorylation contributes to p53 interaction with Bcl2 and Bcl-xL (Park et al., 2005) and that Lys120 acetylation promotes the binding of p53 to Mcl-1 (Sykes et al., 2009). Up to this date, there is no data about p53 mitochondrial targeting in proliferative and living cells.

Monoubiquitylation of p53 was recently described as a possible mechanism for mitochondrial translocation (Marchenko and Moll, 2007). Upon arrival at mitochondria, p53 undergoes deubiquitination by HAUSP, a process essential for complex formation with Bcl-2 family members, because only non-ubiquitinated p53 forms such complexes. The formation of these complexes and consequently the ability of p53 to associate with mitochondria are also conditioned by the status of its binding partners. Notably, post-translational modification of these partners could play an important role; for example, dephosphorylation of Bcl-2 was shown to enhance the formation of the Bcl-2–p53 complex (Deng et al., 2006, Deng et al., 2009). Moreover, non-Bcl-2 proteins could also interfere with mitochondrial binding of p53 and apoptosis induction. Indeed, hepatic IGFBP1 was found to bind to Bak, thus preventing Bak interaction with p53 and apoptosis (Leu et al., 2007). Similarly, ASC (Ohtsuka et al., 2004), clusterin (Zhang et al., 2005) and humanin (Guo et al., 2003) were shown interacting with Bax and Bak and affecting their activation, possibly through the inhibition of their interaction with p53.

Most results concerning the mitochondrial localization of p53 assumed that Bcl-2 family members are crucial mediators of p53 binding to mitochondria, more precisely at the outer membrane, and of its transcription-independent apoptotic activity. However, some studies indicate on the one hand that p53 could localize at a more inner compartment to prevent stress damages and, on the other hand, that p53 could be tightly associated to mitochondria in living and proliferative cells. Clearly, the study of mechanisms which regulate p53 mitochondrial targeting is one of the key areas in the field that requires further work.

8.5 Mitochondrial dynamics and apoptosis

8.5.1 Mitochondrial fission and apoptosis

There is compelling evidence that the eukaryotic organelles, chloroplasts and mitochondria, are evolutionarily derived from bacteria (Gray, 1993, Lutkenhaus, 1998). Mitochondria are dynamic

organelles that continually move, fuse and divide. Thus, the distribution of mitochondria to daughter cells during cell division is an essential feature of cell proliferation and cell survival. These dynamic processes are also believed to ensure an adequate provision of ATP to those cytoplasmic regions where energy consumption is particularly high. Indeed, mitochondria are essential in ensuring ATP production, the usable energy molecule that is required for most of the endoergonic processes (Ernster and Schatz, 1981). This highly efficient process is provided by the oxidative phosphorylation, and allows the generation of an electrochemical gradient across the inner mitochondrial membrane. ATP synthase generates ATP from ADP and phosphate. Tubular mitochondrial network can be also engaged in calcium signaling. Therefore, perturbations of mitochondrial dynamics have tremendous consequences on cell metabolism and therefore on cell life (Benard et al., 2007, Westermann, 2008). In many senescent cell types, an extensive elongation of mitochondria occurs (Mai et al.), and mice defective in mitochondrial fusion cannot sustain development and die (Wakabayashi et al., 2009, Ishihara et al., 2009). Yeast mutants also defective in mitochondrial fusion lose their mitochondrial DNA and cannot run oxidative phosphorylations (Okamoto and Shaw, 2005, Dimmer et al., 2002). Similar perturbations of human cell mitochondria dynamics lead to numerous disorders such as Charcot-Marie-Tooth 2A or 4A or autosomal dominant optic atrophy.

The dynamic nature of mitochondria appears to be dependent upon the cytoskeleton and mechanoenzymes, including kinesins and dynamins (Yoon and McNiven, 2001). Dynamins are a family of GTPases that participate in multiple membrane transport processes, and some of them such as Dnm1p or Mgm1p control mitochondrial morphology. Interestingly, homologs of these GTPases have been identified in higher eukaryotes, including flies, worms and mammalian cells, indicating that this process of mitochondrial morphology maintenance is evolutionarily conserved (Hales and Fuller, 1997, Labrousse et al., 1999, Pitts et al., 1999, Smirnova et al., 1998, Bleazard et al., 1999). Another non-dynamin GTPase called Fzo1p was demonstrated to function in mitochondrial morphogenesis (Yoon and McNiven, 2001). In mammalian cells, at least two proteins, DLP1 and Fis1, are required for fission. The dynamin-related protein DLP1 is a large cytosolic GTPase that is translocated to the mitochondria, where it couples GTP hydrolysis with scission of the mitochondrial tubule (Smirnova et al., 2001). Fis1p is anchored in the outer mitochondrial membrane with its amino-terminus exposed to the cytoplasm and a short carboxy-terminal tail protruding into the mitochondrial intermembrane space (Yoon et al., 2003). When the normal function of DLP1 was inhibited in cultured mammalian cells, mitochondrial tubules became elongated and entangled, collapsing around the nucleus. Further insights have come from work on the *C. elegans* homolog DRP1 showing that DRP1 functions in fission of the mitochondrial outer membrane (MOM).

Post-translational modifications are rapid, effective and reversible ways to regulate protein stability, localization, function, and their interactions with other molecules. Post-translational modifications usually occur as chemical modifications at amino acid residues, including for example,

phosphorylation, SUMOylation, and S-nitrosylation. It has been shown that Drp1 phosphorylation participates in the control of mitochondrial shape integrating cAMP and calcium signals (Taguchi et al., 2007, Chang and Blackstone, 2007, Cribbs and Strack, 2007, Han et al., 2008). The modification of proteins by the small ubiquitin-like modifier (SUMO) is known to regulate an increasing array of cellular processes. Ubc9 and Sumo1 are specific DRP1-interacting proteins and DRP1 is a Sumo1 substrate (Harder et al., 2004). SUMOylation of DRP1 stimulates mitochondrial fission. Furthermore, the mitochondrial-anchored protein ligase (MAPL), the first mitochondrial-anchored SUMO E3 ligase, was shown to link MAPL and the fission machinery (Braschi et al., 2009). Prominent among these post-translational modifications are the addition of ubiquitin moieties that confer new binding surfaces and conformational states on the modified proteins. MARCH5, a mitochondrial E3 ubiquitin ligase has been identified as a molecule that binds mitochondrial Fis1 and mitofusin 2 (Nakamura et al., 2006, Karbowski et al., 2007). MARCH5 RNA interference induces an abnormal elongation and interconnection of mitochondria. Other clues suggest a role of Drp1 in neurodegenerative diseases. Indeed, mutations in PTEN-induced kinase 1 (PINK1) or PARKIN are the most frequent causes of recessive Parkinson's disease. Genetic studies in *Drosophila* indicate that PINK1 acts upstream of Parkin in a common pathway that influences mitochondrial integrity. Thus, the loss of the E3 ubiquitin ligase Parkin or the serine/threonine kinase PINK1 promotes mitochondrial fission and/or inhibits fusion (Poole et al., 2008, Deng et al., 2008). Notably, Pink1 interacts with Drp1, and knocking down Pink1 increases the ratio of mitochondrial fission over fusion proteins, leading to fragmented mitochondria (Cui et al.).

Because it remains unclear how the MOM is permeabilized during apoptosis, several models potentially accounting for MOMP have been put forward. Based on the discovery that fragments of the mitochondrial network associated with remodeling of the cristae are generated during cell death, it has been proposed that the actors of the fission machinery regulate cell death (Bossy-Wetzel et al., 2003, Frank et al., 2001, Karbowski et al., 2002). Interestingly, mitochondrial fragmentation has also been reported during apoptosis of *Drosophila* cells (Goyal et al., 2007, Abdelwahid et al., 2007). However, other observations are disagreeing with the view that mitochondrial fission/fragmentation is important for apoptosis. Studies of mitochondrial dynamics during apoptosis suggest that mitochondrial fragmentation follows, rather than precedes, mitochondrial cytochrome c release after ActD treatment (Arnoult et al., 2005a). Inhibition of *Drp1* expression failed to block apoptosis in response to a number of proapoptotic stimuli (Parone et al., 2006, Estaquier and Arnoult, 2007). Inhibition of *Fis1* expression, another major regulator of mitochondrial fission, has been reported either to inhibit apoptosis (Lee et al., 2004) or to have little effect on this process (Parone et al., 2006). Furthermore, Ced-9, the *C. elegans* Bcl-2 homolog, promotes mitochondrial fusion upon overexpression in mammalian cells, but failed to prevent cytochrome c release or apoptosis (Delivani et al., 2006). Similarly a chemical inhibitor of DRP1, mdivi-1 (for mitochondrial division inhibitor) uncouples

mitochondrial fission and apoptosis (Cassidy-Stone et al., 2008).

8.5.2 Mitochondrial fusion and apoptosis

Mitochondrial fusion in mammalian cells involves a different set of proteins: the large transmembrane GTPase mitofusins (Mfn1, Mfn2) anchored to the MOM and the dynamin-like GTPase OPA1 (optic atrophy 1, Mgm1p) located in the IMS (Ishihara et al., 2004, Chen et al., 2003, Cipolat et al., 2004, Griparic et al., 2004). Despite a high level of homology, Mfn1 and Mfn2 show functional differences. Mfn1 has a center role in mitochondrial docking and fusion, while Mfn2 could participate in the stabilization of the interaction between adjacent mitochondria (Chen et al., 2003, Koshiba et al., 2004). Mice lacking *Mfn1* are viable and display no major defects. On the contrary, *Mfn2* knockout mouse present a degeneration of Purkinje cells (Chen et al., 2007). In yeast, Ugo1p -- an adaptor protein -- is required to couple fusion of the outer membrane, mediated by the Mfns orthologue Fzo1p, and that of the inner membrane, which also requires the Opa1 orthologue Mgm1p (Sesaki and Jensen, 2004). Opa1 is so far the only dynamin-related protein targeted to the inner membrane of mitochondria, via a specific N-terminal targeting sequence, followed by a hydrophobic stretch that allows its insertion into the membrane (Olichon et al., 2002). Mutations in Opa1 are associated with Dominant Optic Atrophy (DOA), the Kjer optic neuropathy, a non-syndromic neurological disease specifically affecting the retinal ganglion cells (RGCs), leading to reduced visual acuity, and sometimes to legal blindness (Delettre et al., 2000, Alexander et al., 2000). Opa1 exists in eight splice variants in humans (five in mouse) (Akepati et al., 2008, Delettre et al., 2001) and is further regulated by posttranslational cleavage that generates short and long forms of the protein. OPA1 contributes to the Inner Mitochondrial Membrane (IMM) structures, i.e. cristae, cristae junction and domains of interaction with the OMM. Strong evidences suggest that OPA1 is required in maintaining respiratory chain integrity. Thus, OPA1 was recently found physically interacting with Complex-I, Complex-II and Complex-III, but not Complex-IV of the respiratory chain, suggesting a possible connection between cristae structure and organization of the respiratory chain C-I to C-III that exchange electrons through the membrane, while electron transfer from C-III to C-IV occurs out of the IMM via cytochrome c (Zanna et al., 2008). Interestingly, fibroblast strains with deleterious OPA1 mutation showed a coupling defect of oxidative phosphorylation as well as a faint decrease in ATP production driven by C-I substrates (Zanna et al., 2008). Therefore, a central OPA1 function consists in maintaining IMM integrity to prevent proton leakage and to facilitate efficient electron transport inside this membrane between respiratory chain complexes. Moreover, OPA1 down-regulation evidenced perturbation of the IMM structure and induces drastic fragmentation of the network (Misaka et al., 2006, Olichon et al., 2007, Legros et al., 2002) that correlated to a strong dissipation of the membrane potential (Olichon et al., 2003, Olichon et al., 2007). OPA1 has been shown to interact with the mitofusins MFN1 and MFN2 (Cipolat et al., 2006, Guillery et al., 2008). In *Caenorhabditis elegans*, mutation in the *eat-3* gene, the *OPA1* orthologue, induces mitochondrial fragmentation, and

shorter and scarce cristae. These worms are smaller, grow slower and show oxidative phosphorylation defects (Kanazawa et al., 2008). In *Drosophila melanogaster*, mutations in *dOpa1* cause haploinsufficiency and heterozygous flies show no discernable phenotype, but a reduced life span (Tang et al., 2009).

Several proteolytic machineries have been implicated in the cleavage of OPA1. Rhomboids are among the most conserved family of polytopic membrane proteins known to date, sequenced in bacterial, archaeal and eukaryotic genomes (Koonin et al., 2003). In mammalian cells, Parl -- for presenilin-associated rhomboid-like protein -- was discovered following a yeast two-hybrid screening using as bait Presenilin-2 (PSEN2), a proapoptotic familial Alzheimer's disease protein (Wolozin et al., 1996). Subsequent studies indicated, however, that the reported interaction between Parl and the presenilin proteins was artifactual (Pellegrini et al., 2001). Parl is localized in the inner mitochondrial membrane, with the N-terminus exposed to the matrix and the C-terminus to the IMS (Jeyaraju et al., 2006). In *S. cerevisiae*, two rhomboid genes exist, *Rbd1* and *Rbd2* (Esser et al., 2002). *Rbd1*, encodes a mitochondrial rhomboid protease, which is required for the processing of cytochrome c peroxidase (Ccp1p). Ablation of *Pcp1/Rbd1* activity has a profound effect on mitochondrial shape. Yeast lacking Pcp1p are missing the 90-kDa short-Mgm1 (OPA1) form but have the 100 kDa long-Mgm1 form (Esser et al., 2002). Mice lacking Parl die between weeks 8 and 12 from cachexia sustained by multisystemic atrophy. However, $Parl^{+/-}$ mice do not show any obvious phenotype, suggesting the existence of compensatory mechanisms to gene dosage effects. Moreover, *Parl* ablation did not alter the morphology of the mitochondrial reticulum or mitochondrial respiration, irrespective of the substrate used by the organelle; loss of *Parl* expression did not affect mitochondria fusion (Cipolat et al., 2006).

Although some groups have proposed that in steady state conditions the function of Parl is to execute the cleavage of Opa1 which is dependent on ATP, to either directly or indirectly liberate an IMS-soluble form of the protein (IMS-Opa1) that assembles in macromolecular complexes with Parl and with the uncleaved IMM-bound form of Opa1 (Herlan et al., 2003, Lemberg et al., 2005, Cipolat et al., 2006), other groups have reported a lack of PARL involvement in OPA1 processing and have implicated other metalloproteases — the m-AAA proteases (matrix of mitochondria oriented; homo- or heteromeric complexes containing paraplegin and/or Afg3L1 and Afg3L2 subunits) and the i-AAA protease Yme1L (intermembrane space oriented) (Griparic et al., 2007, Ishihara et al., 2006, Song et al., 2007). Thus, the bivalent metal chelator, 10-phenanthroline (o-phe) was reported to inhibit m-AAA proteases and OPA1 processing (Ishihara et al., 2006). However, the effects of paraplegin siRNA were modest in preventing OPA1 processing (Duvezin-Caubet et al., 2007). Thus, down-regulation of individual subunits of m-AAA protease isoenzymes did not affect the processing of OPA1. Some confusion may have also arisen from the fact that OPA1 is controlled by complex patterns of alternative splicing and proteolysis. Thus, the scenario proposed is that the i-AAA protease

Yme1L generates the short form of OPA1 (S-OPA1) whereas the remaining isoforms -- the long isoforms of OPA1 (L-OPA1)-- are normally not cleaved by m-AAA proteases. S-and L-OPA1 are both required for fusion. There is also evidence suggesting that a decrease in mitochondrial ATP levels upon inhibition of the F1F0-ATP synthase with oligomycin or upon the dissipation of $\Delta\Psi m$ with the uncoupler CCCP, is crucial in the control of L-OPA1 processing (Baricault et al., 2007). Thus, it has been proposed that another protease mediates processing and degradation of the L-OPA1 isoforms. Two other groups have recently identified another peptidase, OMA1 (Oma1 for overlapping activity with m-AAA protease 1) (Head et al., 2009, Ehses et al., 2009). Mammalian OMA1 is similar in sequence to the yeast mitochondrial Oma1 (Kaser et al., 2003). Interestingly, down-regulation of OMA1 before the addition of CCCP or oligomycin inhibits L-OPA1 processing. However, the implication of OMA1 merits further analysis. Indeed, the substrate specificity of OMA1 is distinct in mammalian and yeast (Duvezin-Caubet et al., 2007). Furthermore, no OMA1-like peptidase can be found in *Caenorhabditis elegans* and *Drosophila melanogaster*, although mitochondrial morphology depends on OPA1 in both organisms. Finally, down-regulation of OMA1 does not affect mitochondrial morphology suggesting that OMA1 is dispensable for the formation of S- and L-OPA1 isoforms (Ehses et al., 2009). Thus, OMA1 should be responsible for stress-induced OPA1 cleavage. Thus, it is likely that several proteases participate in the regulation of OPA1 processing.

Prohibitin complexes assemble with *m*-AAA proteases in the mitochondrial inner membrane, increasing the complexity of protein interaction controlling mitochondrial dynamics (Steglich et al., 1999). PHB complex acts as a chaperone for newly synthesized mitochondrial proteins and is required for a correct yeast replicative lifespan (Coates et al., 1997, Nijtmans et al., 2000). Thus, the sequence similarity of prohibitins to lipid raft–associated proteins of the SPFH family (Browman et al., 2007) is consistent with a scaffolding function of prohibitin complexes in the inner membrane. Multiple copies of two homologous subunits, PHB1 (BAP32, often simply termed prohibitin) and PHB2 (BAP37, REA), form large complexes within the mitochondria (Ikonen et al., 1995, Coates et al., 1997, Berger and Yaffe, 1998). PHB2 is a highly conserved, ubiquitously expressed protein and its homologs are found in bacteria, yeast, plants, *Drosophila* and mammals (Nijtmans et al., 2000). Interestingly, it has been shown an essential role of the prohibitin complex for the processing of OPA1 within mitochondria that results in impaired cell proliferation, resistance toward apoptosis, and mitochondrial cristae morphogenesis. Deletion of *PHB2* leads to the selective loss of L-OPA1 isoforms (Merkwirth et al., 2008). Finally, although prohibitins are required for embryonic development in mice, *Caenorhabditis elegans*, and *Drosophila melanogaster*, deletion of prohibitin genes in yeast leads to premature ageing but does not affect cell survival (Merkwirth et al., 2008). Recently, SLP-2 has been identified as a mitochondrial member of a superfamily of putative scaffolding proteins (Da Cruz et al., 2003, Morrow and Parton, 2005). SLP-2 interacts with MFN2 (Hajek et al., 2007) and with PHB1/2 (Da Cruz et al., 2008). In SLP-2-deficient stressed cells, mitochondria are fragmented, and it has been

proposed that SLP-2 is required for stress-induced mitochondrial hyperfusion (Tondera et al., 2009).

Growing evidences suggest that proteins involved in the control of fusion are also key actors in the control of apoptosis. Thus, fuzzyonions/Mitofusins are reported to antagonize cytochrome c release and to inhibit apoptosis upon transient overexpression (Lee et al., 2004, Sugioka et al., 2004, Jahani-Asl et al., 2007). However, the role of cristae remodeling in the full release of cytochrome c and cell death is also much debated (Parone et al., 2006, Yamaguchi et al., 2008, Sheridan et al., 2008). The downregulation of OPA1 or its cleavage by PARL favors the release of cytochrome c by inducing mitochondrial fragmentation and remodeling of the cristae (Arnoult et al., 2005a, Cipolat et al., 2006, Frezza et al., 2006, Olichon et al., 2003). However, it has been suggested that mitochondrial fission/fragmentation occurs as a consequence of apoptosis-associated permeabilization of MOM and subsequent loss of intermembrane space proteins, such as Opa1, (Arnoult et al., 2005a, Arnoult et al., 2005b). Moreover, Youle and colleagues have found that Bax/Bak-deficient cells show constitutive defects in mitochondrial morphology and contain mitochondria that are shorter than normal (Karbowski et al., 2006). Thus, in these different models mitochondrial fragmentation is uncoupled with apoptosis. OMA1 siRNA slows the onset of apoptosis associated with the absence of L-OPA1 processing. This preventive effect on OPA1 cleavage was stronger for ActD than for Staurosporine-mediated apoptosis. Intriguingly, OMA1 siRNA prevents cytochrome C release and cell death following ActD treatment. This may appear paradoxical given that loss of OPA1 is more a consequence of MOMP-mediated by ActD (Sheridan et al., 2008). Like *OPA1* knockout cells, *PHB2* deficient cells are more sensitive to apoptotic stimuli involving both the intrinsic and the extrinsic pathways (Merkwirth et al., 2008). Altogether, these observations suggest that mitochondrial fragmentation is not the main driving force *per se*, but the absence of or defect in those proteins within the mitochondria increases mitochondria sensitivity to apoptotic insults.

Because as mentioned Bax/Bak are the gate keepers controlling mitochondria, it remains unclear whether Bax/Bak is required for mitochondrial fragmentation, given that this process is systematically reported during Bax/Bak-dependent apoptosis (Arnoult et al., 2005a, Parone et al., 2006, Yamaguchi et al., 2008, Sheridan et al., 2008). Several groups have described Bax/Bak-independent release of cytochrome c (Scorrano et al., 2003, Claveria et al., 2004, Lei et al., 2006, Majewski et al., 2004, Mizuta et al., 2007). Thus, whether defective expression of *OPA1* or *PHB2* sensitizes cells to apoptotic insults that do not depend on Bax and Bak proteins could be an interesting question.

8.6 Concluding remarks

In conclusion, mitochondria appears today at the heart of apoptosis signaling. However, although MOMP appears as a decisive step during mammalian cells apoptosis, the role of mitochondria in nematodes and flies remains more elusive. Moreover, in spite of the growing amount of data

concerning the mechanisms and regulations of MOMP in mammals, several concurrent non-exclusive hypotheses still coexist and a unifying model (if pertinent) remains to be established. Mechanisms involved in Bcl-2 family proteins mitochondrial translocation and activation, as well as their relationship with other proteins constitutively or transiently located to mitochondria that are critical for the survival/death behavior of cell populations remain to be fully understood. The answers to these questions should allow designing new chemotherapeutic drugs for cancer and other apoptosis-related diseases treatment.

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8.8 References

ABDELWAHID, E., YOKOKURA, T., KRIESER, R. J., BALASUNDARAM, S., FOWLE, W. H. & WHITE, K. 2007. Mitochondrial disruption in Drosophila apoptosis. *Dev Cell,* 12**,** 793-806.

ACHANTA, G., SASAKI, R., FENG, L., CAREW, J. S., LU, W., PELICANO, H., KEATING, M. J. & HUANG, P. 2005. Novel role of p53 in maintaining mitochondrial genetic stability through interaction with DNA Pol gamma. *Embo J,* 24**,** 3482-92.

AKEPATI, V. R., MULLER, E. C., OTTO, A., STRAUSS, H. M., PORTWICH, M. & ALEXANDER, C. 2008. Characterization of OPA1 isoforms isolated from mouse tissues. *J Neurochem,* 106**,** 372-83.

AKHTAR, R. S., GENG, Y., KLOCKE, B. J. & ROTH, K. A. 2006. Neural precursor cells possess multiple p53-dependent apoptotic pathways. *Cell Death Differ,* 13**,** 1727-39.

ALADJEM, M. I., SPIKE, B. T., RODEWALD, L. W., HOPE, T. J., KLEMM, M., JAENISCH, R. & WAHL, G. M. 1998. ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage. *Curr Biol,* 8**,** 145-55.

ALEXANDER, C., VOTRUBA, M., PESCH, U. E., THISELTON, D. L., MAYER, S., MOORE, A., RODRIGUEZ, M., KELLNER, U., LEO-KOTTLER, B., AUBURGER, G., BHATTACHARYA, S. S. & WISSINGER, B. 2000. OPA1, encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. *Nat Genet,* 26**,** 211-5.

ARNOULT, D., GRODET, A., LEE, Y. J., ESTAQUIER, J. & 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**,** 35742-50.

ARNOULT, D., RISMANCHI, N., GRODET, A., ROBERTS, R. G., SEEBURG, D. P., ESTAQUIER, J., SHENG, M. & BLACKSTONE, C. 2005b. Bax/Bak-dependent release of DDP/TIMM8a promotes Drp1-mediated mitochondrial fission and mitoptosis during programmed cell death. *Curr Biol,* 15**,** 2112-8.

AUTRET, A. & MARTIN, S. J. 2009. Emerging role for members of the Bcl-2 family in mitochondrial morphogenesis. *Mol Cell,* 36**,** 355-63.

BAO, Q. & SHI, Y. 2007. Apoptosome: a platform for the activation of initiator caspases. *Cell Death Differ,* 14**,** 56-65.

BARICAULT, L., SEGUI, B., GUEGAND, L., OLICHON, A., VALETTE, A., LARMINAT, F. & LENAERS, G. 2007. OPA1 cleavage depends on decreased mitochondrial ATP level and bivalent metals. *Exp Cell Res,* 313**,** 3800-8.

BECKER, T., GEBERT, M., PFANNER, N. & VAN DER LAAN, M. 2009. Biogenesis of mitochondrial membrane proteins. *Curr Opin Cell Biol,* 21**,** 484-93.

BELLOT, G., CARTRON, P. F., ER, E., OLIVER, L., JUIN, P., ARMSTRONG, L. C., BORNSTEIN, P., MIHARA, K., MANON, S. & VALLETTE, F. M. 2007. TOM22, a core component of the mitochondria outer membrane protein translocation pore, is a mitochondrial receptor for the proapoptotic protein Bax. *Cell Death Differ,* 14**,** 785-94.

BENARD, G., BELLANCE, N., JAMES, D., PARRONE, P., FERNANDEZ, H., LETELLIER, T. & ROSSIGNOL, R. 2007. Mitochondrial bioenergetics and structural network organization. *J Cell Sci,* 120**,** 838-48.

BENSAAD, K. & VOUSDEN, K. H. 2007. p53: new roles in metabolism. *Trends Cell Biol,* 17**,** 286-91.

BERGER, K. H. & YAFFE, M. P. 1998. Prohibitin family members interact genetically with mitochondrial inheritance components in Saccharomyces cerevisiae. *Mol Cell Biol,* 18**,** 4043-52.

BERGMANN, A. 2010. The role of ubiquitylation for the control of cell death in Drosophila. *Cell Death Differ,* 17**,** 61-7.

BLEAZARD, W., MCCAFFERY, J. M., KING, E. J., BALE, S., MOZDY, A., TIEU, Q., NUNNARI, J. & SHAW, J. M. 1999. The dynamin-related GTPase Dnm1 regulates mitochondrial fission in yeast. *Nat Cell Biol,* 1**,** 298-304.

BORNER, C., MARTINOU, I., MATTMANN, C., IRMLER, M., SCHAERER, E., MARTINOU, J. C. & TSCHOPP, J. 1994. The protein bcl-2 alpha does not require membrane attachment, but two conserved domains to suppress apoptosis. *J. Cell Biol.,* 126**,** 1059-1068.

BOSARI, S., VIALE, G., RONCALLI, M., GRAZIANI, D., BORSANI, G., LEE, A. K. & COGGI, G. 1995. p53 gene mutations, p53 protein accumulation and compartmentalization in colorectal adenocarcinoma. *Am J Pathol,* 147**,** 790-8.

BOSSY-WETZEL, E., BARSOUM, M. J., GODZIK, A., SCHWARZENBACHER, R. & LIPTON, S. A. 2003. Mitochondrial fission in apoptosis, neurodegeneration and aging. *Curr Opin Cell Biol,* 15**,** 706-16.

BRACHMANN, C. B., JASSIM, O. W., WACHSMUTH, B. D. & CAGAN, R. L. 2000. The Drosophila bcl-2 family member dBorg-1 functions in the apoptotic response to UV-irradiation. *Curr Biol,* 10**,** 547-50.

BRADY, G. F. & DUCKETT, C. S. 2009. A caspase homolog keeps CED-3 in check. *Trends Biochem Sci,* 34**,** 104-7.

BRASCHI, E., ZUNINO, R. & MCBRIDE, H. M. 2009. MAPL is a new mitochondrial SUMO E3 ligase that regulates mitochondrial fission. *EMBO Rep,* 10**,** 748-54.

BREDESEN, D. E., RAO, R. V. & MEHLEN, P. 2006. Cell death in the nervous system. *Nature,* 443**,** 796-802.

BROOKS, C. L. & GU, W. 2006. p53 ubiquitination: Mdm2 and beyond. *Mol Cell,* 21**,** 307-15.

BROWMAN, D. T., HOEGG, M. B. & ROBBINS, S. M. 2007. The SPFH domain-containing proteins: more than lipid raft markers. *Trends Cell Biol,* 17**,** 394-402.

BRUN, S., RINCHEVAL, V., GAUMER, S., MIGNOTTE, B. & GUENAL, I. 2002. reaper and bax initiate two different apoptotic pathways affecting mitochondria and antagonized by bcl-2 in Drosophila. *Oncogene,* 21**,** 6458-70.

CAELLES, C., HELMBERG, A. & KARIN, M. 1994. p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. *Nature,* 370**,** 220-223.

CARTRON, P. F., BELLOT, G., OLIVER, L., GRANDIER-VAZEILLE, X., MANON, S. & VALLETTE, F. M. 2008. Bax inserts into the mitochondrial outer membrane by different mechanisms. *FEBS Lett,* 582**,** 3045-51.

CARTRON, P. F., GALLENNE, T., BOUGRAS, G., GAUTIER, F., MANERO, F., VUSIO, P., MEFLAH, K., VALLETTE, F. M. & JUIN, P. 2004. The first alpha helix of Bax plays a necessary role in its ligand-induced activation by the BH3-only proteins Bid and PUMA. *Mol Cell,* 16**,** 807-18.

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. & 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.

CHAI, J., YAN, N., HUH, J. R., WU, J. W., LI, W., HAY, B. A. & SHI, Y. 2003. Molecular mechanism of Reaper-Grim-Hid-mediated suppression of DIAP1-dependent Dronc ubiquitination. *Nat Struct Biol,* 10**,** 892-8. .

CHANG, C. R. & BLACKSTONE, C. 2007. Cyclic AMP-dependent protein kinase phosphorylation of Drp1 regulates its GTPase activity and mitochondrial morphology. *J Biol Chem,* 282**,** 21583-7.

CHEN, D., YU, Z., ZHU, Z. & LOPEZ, C. D. 2006. The p53 pathway promotes efficient mitochondrial DNA base excision repair in colorectal cancer cells. *Cancer Res,* 66**,** 3485-94.

CHEN, H., DETMER, S. A., EWALD, A. J., GRIFFIN, E. E., FRASER, S. E. & 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., MCCAFFERY, J. M. & CHAN, D. C. 2007. Mitochondrial fusion protects against neurodegeneration in the cerebellum. *Cell,* 130**,** 548-62.

CHEN, Z. X. & PERVAIZ, S. 2009a. BCL-2: pro-or anti-oxidant? *Front Biosci (Elite Ed),* 1**,** 263-8.

CHEN, Z. X. & PERVAIZ, S. 2009b. Involvement of cytochrome c oxidase subunits Va and Vb in the regulation of cancer cell metabolism by Bcl-2. *Cell Death Differ***,** doi: 10.1038/cdd.2009.132.

CHENG, E. H., SHEIKO, T. V., FISHER, J. K., CRAIGEN, W. J. & KORSMEYER, S. J. 2003. VDAC2 inhibits BAK activation and mitochondrial apoptosis. *Science,* 301**,** 513-7.

CHIPUK, J. E., BOUCHIER-HAYES, L., KUWANA, T., NEWMEYER, D. D. & GREEN, D. R. 2005. PUMA couples the nuclear and cytoplasmic proapoptotic function of p53. *Science,* 309**,** 1732-5.

CHIPUK, J. E., KUWANA, T., BOUCHIER-HAYES, L., DROIN, N. M., NEWMEYER, D. D., SCHULER, M. & GREEN, D. R. 2004. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science,* 303**,** 1010-4.

CHIPUK, J. E., MOLDOVEANU, T., LLAMBI, F., PARSONS, M. J. & GREEN, D. R. The BCL-2 family reunion. *Mol Cell,* 37**,** 299-310.

CHOU, C. H., LEE, R. S. & YANG-YEN, H. F. 2006. An internal EELD domain facilitates mitochondrial targeting of Mcl-1 via a Tom70-dependent pathway. *Mol Biol Cell,* 17**,** 3952-63.

CIPOLAT, S., MARTINS DE BRITO, O., DAL ZILIO, B. & SCORRANO, L. 2004. OPA1 requires mitofusin 1 to promote mitochondrial fusion. *Proc Natl Acad Sci U S A,* 101**,** 15927-32.

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., PELLEGRINI, L., D'HOOGE, R., SCORRANO, L. & DE STROOPER, B. 2006. Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. *Cell,* 126**,** 163-75.

CLARKE, P. G. 1990. Developmental cell death: morphological diversity and multiple mechanisms. *Anat Embryol (Berl),* 181**,** 195-213.

CLARKE, P. G. H. & CLARKE, S. 1996. Nineteenth century research on naturally occuring cell death and related phenomena. *Anat. Embryol.,* 193**,** 81-99.

CLAVERIA, C., CAMINERO, E., MARTINEZ, A. C., CAMPUZANO, S. & TORRES, M. 2002. GH3, a novel proapoptotic domain in *Drosophila* Grim, promotes a mitochondrial death pathway. *Embo J.,* 21**,** 3327-3336.

CLAVERIA, C., MARTINEZ, A. C. & TORRES, M. 2004. A Bax/Bak-independent mitochondrial death pathway triggered by *Drosophila* Grim GH3 domain in mammalian cells. *J Biol Chem,* 279**,** 1368-75.

COATES, P. J., JAMIESON, D. J., SMART, K., PRESCOTT, A. R. & HALL, P. A. 1997. The prohibitin family of mitochondrial proteins regulate replicative lifespan. *Curr Biol,* 7**,** 607-10.

COLIN, J., GARIBAL, J., MIGNOTTE, B. & GUENAL, I. 2009a. The mitochondrial TOM complex modulates bax-induced apoptosis in Drosophila. *Biochem Biophys Res Commun,* 379**,** 939-943.

COLIN, J., GAUMER, S., GUENAL, I. & MIGNOTTE, B. 2009b. Mitochondria, Bcl-2 family proteins and apoptosomes: of worms, flies and men. *Front Biosci,* 14**,** 4127-37.

COLUSSI, P. A., QUINN, L. M., HUANG, D. C., COOMBE, M., READ, S. H., RICHARDSON, H. & KUMAR, S. 2000. Debcl, a proapoptotic Bcl-2 homologue, is a component of the Drosophila melanogaster cell death machinery. *J Cell Biol,* 148**,** 703-14.

CRIBBS, J. T. & STRACK, S. 2007. Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. *EMBO Rep,* 8**,** 939-44.

CUI, M., TANG, X., CHRISTIAN, W. V., YOON, Y. & TIEU, K. Perturbations in mitochondrial dynamics induced by human mutant PINK1 can be rescued by the mitochondrial division inhibitor mdivi-1. *J Biol Chem*.

DA CRUZ, S., PARONE, P. A., GONZALO, P., BIENVENUT, W. V., TONDERA, D., JOURDAIN, A., QUADRONI, M. & MARTINOU, J. C. 2008. SLP-2 interacts with prohibitins in the mitochondrial inner membrane and contributes to their stability. *Biochim Biophys Acta,* 1783**,** 904-11.

DA CRUZ, S., XENARIOS, I., LANGRIDGE, J., VILBOIS, F., PARONE, P. A. & MARTINOU, J. C. 2003. Proteomic analysis of the mouse liver mitochondrial inner membrane. *J Biol Chem,* 278**,** 41566-71.

DAISH, T. J., MILLS, K. & KUMAR, S. 2004. *Drosophila* caspase DRONC is required for specific developmental cell death pathways and stress-induced apoptosis. *Dev Cell,* 7**,** 909-15.

DANIAL, N. N. 2008. BAD: undertaker by night, candyman by day. *Oncogene,* 27 Suppl 1**,** S53-70.

DANIAL, N. N., GRAMM, C. F., SCORRANO, L., ZHANG, C. Y., KRAUSS, S., RANGER, A. M., DATTA, S. R., GREENBERG, M. E., LICKLIDER, L. J., LOWELL, B. B., GYGI, S. P. & KORSMEYER, S. J. 2003. BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis. *Nature,* 424**,** 952-6.

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., DEENEY, J. T., ROBERTSON, K., MORASH, J., KULKARNI, A., NESCHEN, S., KIM, S., GREENBERG, M. E., CORKEY, B. E., SHIRIHAI, O. S., SHULMAN, G. I., LOWELL, B. B. & KORSMEYER, S. J. 2008. Dual role of proapoptotic BAD in insulin secretion and beta cell survival. *Nat Med,* 14**,** 144-53.

DELETTRE, C., GRIFFOIN, J. M., KAPLAN, J., DOLLFUS, H., LORENZ, B., FAIVRE, L., LENAERS, G., BELENGUER, P. & HAMEL, C. P. 2001. Mutation spectrum and splicing variants in the OPA1 gene. *Hum Genet,* 109**,** 584-91.

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. & HAMEL, C. P. 2000. Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet,* 26**,** 207-10.

DELIVANI, P., ADRAIN, C., TAYLOR, R. C., DURIEZ, P. J. & MARTIN, S. J. 2006. Role for CED-9 and Egl-1 as regulators of mitochondrial fission and fusion dynamics. *Mol Cell,* 21**,** 761-73.

DENG, H., DODSON, M. W., HUANG, H. & GUO, M. 2008. The Parkinson's disease genes pink1 and parkin promote mitochondrial fission and/or inhibit fusion in Drosophila. *Proc Natl Acad Sci U S A,* 105**,** 14503-8.

DENG, X., GAO, F., FLAGG, T., ANDERSON, J. & MAY, W. S. 2006. Bcl2's flexible loop domain regulates p53 binding and survival. *Mol Cell Biol,* 26**,** 4421-34.

DENG, X., GAO, F. & MAY, W. S. 2009. Protein phosphatase 2A inactivates Bcl2's antiapoptotic function by dephosphorylation and up-regulation of Bcl2-p53 binding. *Blood,* 113**,** 422-8.

DESAGHER, S. & MARTINOU, J. C. 2000. Mitochondria as the central control point of apoptosis. *Trends Cell Biol,* 10**,** 369-77.

DIMMER, K. S., FRITZ, S., FUCHS, F., MESSERSCHMITT, M., WEINBACH, N., NEUPERT, W. & WESTERMANN, B. 2002. Genetic basis of mitochondrial function and morphology in Saccharomyces cerevisiae. *Mol Biol Cell,* 13**,** 847-53.

DONAHUE, R. J., RAZMARA, M., HOEK, J. B. & KNUDSEN, T. B. 2001. Direct influence of the p53 tumor suppressor on mitochondrial biogenesis and function. *FASEB J,* 15**,** 635-44.

DU, C., FANG, M., LI, Y., LI, L. & WANG, X. 2000. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell,* 102**,** 33-42.

DUVEZIN-CAUBET, S., KOPPEN, M., WAGENER, J., ZICK, M., ISRAEL, L., BERNACCHIA, A., JAGASIA, R., RUGARLI, E. I., IMHOF, A., NEUPERT, W., LANGER, T. & REICHERT, A. S. 2007. OPA1 processing reconstituted in yeast depends on the subunit composition of the m-AAA protease in mitochondria. *Mol Biol Cell,* 18**,** 3582-90.

EHSES, S., RASCHKE, I., MANCUSO, G., BERNACCHIA, A., GEIMER, S., TONDERA, D., MARTINOU, J. C., WESTERMANN, B., RUGARLI, E. I. & LANGER, T. 2009. Regulation of OPA1 processing and mitochondrial fusion by m-AAA protease isoenzymes and OMA1. *J Cell Biol,* 187**,** 1023-36.

ELLIS, H. M. & HORVITZ, H. R. 1986. Genetic control of programmed cell death in the nematode *C. elegans*. *Cell,* 44**,** 817-829.

ERNSTER, L. & SCHATZ, G. 1981. Mitochondria: a historical review. *J Cell Biol,* 91**,** 227s-255s.

ERSTER, S., MIHARA, M., KIM, R. H., PETRENKO, O. & MOLL, U. M. 2004. In vivo mitochondrial p53 translocation triggers a rapid first wave of cell death in response to DNA damage that can precede p53 target gene activation. *Mol Cell Biol,* 24**,** 6728-41.

ESSER, K., TURSUN, B., INGENHOVEN, M., MICHAELIS, G. & PRATJE, E. 2002. A novel two-step mechanism for removal of a mitochondrial signal sequence involves the mAAA complex and the putative rhomboid protease Pcp1. *J Mol Biol,* 323**,** 835-43.

ESSMANN, F., POHLMANN, S., GILLISSEN, B., DANIEL, P. T., SCHULZE-OSTHOFF, K. & JANICKE, R. U. 2005. Irradiation-induced translocation of p53 to mitochondria in the absence of apoptosis. *J Biol Chem,* 280**,** 37169-77.

ESTAQUIER, J. & ARNOULT, D. 2007. Inhibiting Drp1-mediated mitochondrial fission selectively prevents the release of cytochrome c during apoptosis. *Cell Death Differ,* 14**,** 1086-94.

FERECATU, I., BERGEAUD, M., RODRIGUEZ-ENFEDAQUE, A., LE FLOCH, N., OLIVER, L., RINCHEVAL, V., RENAUD, F., VALLETTE, F. M., MIGNOTTE, B. & VAYSSIERE, J. L. 2009. Mitochondrial localization of the low level p53 protein in proliferative cells. *Biochem Biophys Res Commun,* 387**,** 772-7.

FLETCHER, J. I., MEUSBURGER, S., HAWKINS, C. J., RIGLAR, D. T., LEE, E. F., FAIRLIE, W. D., HUANG, D. C. & ADAMS, J. M. 2008. Apoptosis is triggered when prosurvival Bcl-2 proteins cannot restrain Bax. *Proc Natl Acad Sci U S A,* 105**,** 18081-7.

FLEURY, C., MIGNOTTE, B. & VAYSSIERE, J. L. 2002. Mitochondrial reactive oxygen species in cell death signaling. *Biochimie,* 84**,** 131-41.

FRANK, S., GAUME, B., BERGMANN-LEITNER, E. S., LEITNER, W. W., ROBERT, E. G., CATEZ, F., SMITH, C. L. & YOULE, R. J. 2001. The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. *Dev Cell,* 1**,** 515-25.

FREEL, C. D., RICHARDSON, D. A., THOMENIUS, M. J., GAN, E. C., HORN, S. R., OLSON, M. R. & KORNBLUTH, S. 2008. Mitochondrial localization of Reaper to promote inhibitors of apoptosis protein degradation conferred by GH3 domain-lipid interactions. *J Biol Chem,* 283**,** 367-79.

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. & SCORRANO, L. 2006. OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell,* 126**,** 177-89.

FRIDMAN, J. S. & LOWE, S. W. 2003. Control of apoptosis by p53. *Oncogene,* 22**,** 9030-40.

GALINDO, K. A., LU, W. J., PARK, J. H. & ABRAMS, J. M. 2009. The Bax/Bak ortholog in Drosophila, Debcl, exerts limited control over programmed cell death. *Development,* 136**,** 275-83.

GAUMER, S., GUENAL, I., BRUN, S., THEODORE, L. & MIGNOTTE, B. 2000. Bcl-2 and Bax mammalian regulators of apoptosis are functional in Drosophila. *Cell Death Differ,* 7**,** 804-14.

GENG, Y., AKHTAR, R. S., SHACKA, J. J., KLOCKE, B. J., ZHANG, J., CHEN, X. & ROTH, K. A. 2007. p53 transcription-dependent and -independent regulation of cerebellar neural precursor cell apoptosis. *J Neuropathol Exp Neurol,* 66**,** 66-74.

GEYER, R. K., YU, Z. K. & MAKI, C. G. 2000. The MDM2 RING-finger domain is required to promote p53 nuclear export. *Nat Cell Biol,* 2**,** 569-73.

GIAM, M., HUANG, D. C. & BOUILLET, P. 2008. BH3-only proteins and their roles in programmed cell death. *Oncogene,* 27 Suppl 1**,** S128-36.

GIANNAKAKOU, P., SACKETT, D. L., WARD, Y., WEBSTER, K. R., BLAGOSKLONNY, M. V. & FOJO, T. 2000. p53 is associated with cellular microtubules and is transported to the nucleus by dynein. *Nat Cell Biol,* 2**,** 709-17.

GODEFROY, N., LEMAIRE, C., RENAUD, F., RINCHEVAL, V., PEREZ, S., PARVU-FERECATU, I., MIGNOTTE, B. & VAYSSIERE, J. L. 2004. p53 can promote mitochondria- and caspase-independent apoptosis. *Cell Death Differ,* 11**,** 785-7.

GOTTIFREDI, V. & PRIVES, C. 2001. Molecular biology. Getting p53 out of the nucleus. *Science,* 292**,** 1851-2.

GOYAL, G., FELL, B., SARIN, A., YOULE, R. J. & SRIRAM, V. 2007. Role of mitochondrial remodeling in programmed cell death in Drosophila melanogaster. *Dev Cell,* 12**,** 807-16.

GOYAL, L., MCCALL, K., AGAPITE, J., HARTWIEG, E. & STELLER, H. 2000. Induction of apoptosis by *Drosophila reaper, hid* and *grim* through inhibition of IAP function. *Embo J.,* 19**,** 589-597.

GRAY, M. W. 1993. Origin and evolution of organelle genomes. *Curr. Opin. Genet. Dev.,* 3**,** 884-890.

GREEN, D. R. & KROEMER, G. 2009. Cytoplasmic functions of the tumour suppressor p53. *Nature,* 458**,** 1127-30.

GRINBERG, M., SCHWARZ, M., ZALTSMAN, Y., EINI, T., NIV, H., PIETROKOVSKI, S. & GROSS, A. 2005. Mitochondrial carrier homolog 2 is a target of tBID in cells signaled to die by tumor necrosis factor alpha. *Mol Cell Biol,* 25**,** 4579-90.

GRIPARIC, L., KANAZAWA, T. & VAN DER BLIEK, A. M. 2007. Regulation of the mitochondrial dynamin-like protein Opa1 by proteolytic cleavage. *J Cell Biol,* 178**,** 757-64.

GRIPARIC, L., VAN DER WEL, N. N., OROZCO, I. J., PETERS, P. J. & 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**,** 18792-8.

GROSS, A. 2006. BID as a double agent in cell life and death. *Cell Cycle,* 5**,** 582-4.

GROSSMAN, S. R., DEATO, M. E., BRIGNONE, C., CHAN, H. M., KUNG, A. L., TAGAMI, H., NAKATANI, Y. & LIVINGSTON, D. M. 2003. Polyubiquitination of p53 by a ubiquitin ligase activity of p300. *Science,* 300**,** 342-4.

GUICCIARDI, M. E. & GORES, G. J. 2009. Life and death by death receptors. *FASEB J,* 23**,** 1625-37.

GUILLERY, O., MALKA, F., LANDES, T., GUILLOU, E., BLACKSTONE, C., LOMBES, A., BELENGUER, P., ARNOULT, D. & ROJO, M. 2008. Metalloprotease-mediated OPA1 processing is modulated by the mitochondrial membrane potential. *Biol Cell,* 100**,** 315-25.

GUO, B., ZHAI, D., CABEZAS, E., WELSH, K., NOURAINI, S., SATTERTHWAIT, A. C. & REED, J. C. 2003. Humanin peptide suppresses apoptosis by interfering with Bax activation. *Nature,* 423**,** 456-61.

HAJEK, P., CHOMYN, A. & ATTARDI, G. 2007. Identification of a novel mitochondrial complex containing mitofusin 2 and stomatin-like protein 2. *J Biol Chem,* 282**,** 5670-81.

HALES, K. G. & FULLER, M. T. 1997. Developmentally regulated mitochondrial fusion mediated by a conserved, novel, predicted GTPase. *Cell,* 90**,** 121-9.

HAN, X. J., LU, Y. F., LI, S. A., KAITSUKA, T., SATO, Y., TOMIZAWA, K., NAIRN, A. C., TAKEI, K., MATSUI, H. & MATSUSHITA, M. 2008. CaM kinase I alpha-induced phosphorylation of Drp1 regulates mitochondrial morphology. *J Cell Biol,* 182**,** 573-85.

HARDER, Z., ZUNINO, R. & MCBRIDE, H. 2004. Sumo1 conjugates mitochondrial substrates and participates in mitochondrial fission. *Curr Biol,* 14**,** 340-5.

HAUPT, Y., ROWAN, S., SHAULIAN, E., VOUSDEN, K. H. & OREN, M. 1995. Induction of apoptosis in HeLa cells by trans-activation-deficient p53. *Genes Dev.,* 9**,** 2170-2183.

HEAD, B., GRIPARIC, L., AMIRI, M., GANDRE-BABBE, S. & VAN DER BLIEK, A. M. 2009. Inducible proteolytic inactivation of OPA1 mediated by the OMA1 protease in mammalian cells. *J Cell Biol,* 187**,** 959-66.

HEGDE, R., SRINIVASULA, S. M., ZHANG, Z., WASSELL, R., MUKATTASH, R., CILENTI, L., DUBOIS, G., LAZEBNIK, Y., ZERVOS, A. S., FERNANDES-ALNEMRI, T. & ALNEMRI,

E. S. 2002. Identification of Omi/HtrA2 as a mitochondrial apoptotic serine protease that disrupts inhibitor of apoptosis protein-caspase interaction. *J Biol Chem,* 277**,** 432-8.

HENGARTNER, M. O. & HORVITZ, H. R. 1994. *C. elegans* cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene *bcl-2*. *Cell,* 76**,** 665-676.

HERLAN, M., VOGEL, F., BORNHOVD, C., NEUPERT, W. & REICHERT, A. S. 2003. Processing of Mgm1 by the rhomboid-type protease Pcp1 is required for maintenance of mitochondrial morphology and of mitochondrial DNA. *J Biol Chem,* 278**,** 27781-8.

HO, P. K. & HAWKINS, C. J. 2005. Mammalian initiator apoptotic caspases. *FEBS J,* 272**,** 5436-53.

HOFFMAN, W. H., BIADE, S., ZILFOU, J. T., CHEN, J. & MURPHY, M. 2002. Transcriptional repression of the anti-apoptotic survivin gene by wild type p53. *J. Biol. Chem.,* 277**,** 3247-3257.

HUGUNIN, M., QUINTAL, L. J., MANKOVICH, J. A. & GHAYUR, T. 1996. Protease activity of in vitro transcribed and translated Caenorhabditis elegans cell death gene (ced-3) product. *J Biol Chem,* 271**,** 3517-22.

IGAKI, T., KANUKA, H., INOHARA, N., SAWAMOTO, K., NUNEZ, G., OKANO, H. & MIURA, M. 2000. Drob-1, a Drosophila member of the Bcl-2/CED-9 family that promotes cell death. *Proc Natl Acad Sci U S A,* 97**,** 662-7.

IKONEN, E., FIEDLER, K., PARTON, R. G. & SIMONS, K. 1995. Prohibitin, an antiproliferative protein, is localized to mitochondria. *FEBS Lett,* 358**,** 273-7.

ISHIHARA, N., EURA, Y. & MIHARA, K. 2004. Mitofusin 1 and 2 play distinct roles in mitochondrial fusion reactions via GTPase activity. *J Cell Sci,* 117**,** 6535-46.

ISHIHARA, N., FUJITA, Y., OKA, T. & MIHARA, K. 2006. Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. *Embo J,* 25**,** 2966-77.

ISHIHARA, N., NOMURA, M., JOFUKU, A., KATO, H., SUZUKI, S. O., MASUDA, K., OTERA, H., NAKANISHI, Y., NONAKA, I., GOTO, Y., TAGUCHI, N., MORINAGA, H., MAEDA, M., TAKAYANAGI, R., YOKOTA, S. & MIHARA, K. 2009. Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. *Nat Cell Biol,* 11**,** 958-66.

JAHANI-ASL, A., CHEUNG, E. C., NEUSPIEL, M., MACLAURIN, J. G., FORTIN, A., PARK, D. S., MCBRIDE, H. M. & SLACK, R. S. 2007. Mitofusin 2 protects cerebellar granule neurons against injury-induced cell death. *J Biol Chem,* 282**,** 23788-98.

JEYARAJU, D. V., XU, L., LETELLIER, M. C., BANDARU, S., ZUNINO, R., BERG, E. A., MCBRIDE, H. M. & PELLEGRINI, L. 2006. Phosphorylation and cleavage of presenilin-associated rhomboid-like protein (PARL) promotes changes in mitochondrial morphology. *Proc Natl Acad Sci U S A,* 103**,** 18562-7.

JIANG, P., DU, W., HEESE, K. & WU, M. 2006. The Bad guy cooperates with good cop p53: Bad is transcriptionally up-regulated by p53 and forms a Bad/p53 complex at the mitochondria to induce apoptosis. *Mol Cell Biol,* 26**,** 9071-82.

JOHNSON, T. M., MEADE, K., PATHAK, N., MARQUES, M. R. & ATTARDI, L. D. 2008. Knockin mice expressing a chimeric p53 protein reveal mechanistic differences in how p53 triggers apoptosis and senescence. *Proc Natl Acad Sci U S A,* 105**,** 1215-20.

JOURDAIN, A. & MARTINOU, J. C. 2009. Mitochondrial outer-membrane permeabilization and remodelling in apoptosis. *Int J Biochem Cell Biol,* 41**,** 1884-9.

KAKUDO, Y., SHIBATA, H., OTSUKA, K., KATO, S. & ISHIOKA, C. 2005. Lack of correlation between p53-dependent transcriptional activity and the ability to induce apoptosis among 179 mutant p53s. *Cancer Res,* 65**,** 2108-14.

KANAZAWA, T., ZAPPATERRA, M. D., HASEGAWA, A., WRIGHT, A. P., NEWMAN-SMITH, E. D., BUTTLE, K. F., MCDONALD, K., MANNELLA, C. A. & VAN DER BLIEK, A. M. 2008. The C. elegans Opa1 homologue EAT-3 is essential for resistance to free radicals. *PLoS Genet,* 4**,** e1000022.

KARBOWSKI, M., LEE, Y. J., GAUME, B., JEONG, S. Y., FRANK, S., NECHUSHTAN, A., SANTEL, A., FULLER, M., SMITH, C. L. & YOULE, R. J. 2002. Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis. *J Cell Biol,* 159**,** 931-8.

KARBOWSKI, M., NEUTZNER, A. & YOULE, R. J. 2007. The mitochondrial E3 ubiquitin ligase MARCH5 is required for Drp1 dependent mitochondrial division. *J Cell Biol,* 178**,** 71-84.

KARBOWSKI, M., NORRIS, K. L., CLELAND, M. M., JEONG, S. Y. & YOULE, R. J. 2006. Role of Bax and Bak in mitochondrial morphogenesis. *Nature,* 443**,** 658-62.

KASER, M., KAMBACHELD, M., KISTERS-WOIKE, B. & LANGER, T. 2003. Oma1, a novel membrane-bound metallopeptidase in mitochondria with activities overlapping with the m-AAA protease. *J Biol Chem,* 278**,** 46414-23.

KATSUMOTO, T., HIGAKI, K., OHNO, K. & ONODERA, K. 1995. Cell-cycle dependent biosynthesis and localization of p53 protein in untransformed human cells. *Biol Cell,* 84**,** 167-73.

KAWAGUCHI, Y., ITO, A., APPELLA, E. & YAO, T. P. 2006. Charge modification at multiple C-terminal lysine residues regulates p53 oligomerization and its nucleus-cytoplasm trafficking. *J Biol Chem,* 281**,** 1394-400.

KERR, J. F., WYLLIE, A. H. & CURRIE, A. R. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer,* 26**,** 239-57.

KERR, J. F. R. & HARMON, B. V. 1991. Definition and incidence of apoptosis: an historical perspective. In apoptosis: the molecular basis of cell death, L. D. Tomei and F. O. Cope, eds. *(New York: Cold spring harbor laboratory press)***,** 5-29.

KIM, H., TU, H. C., REN, D., TAKEUCHI, O., JEFFERS, J. R., ZAMBETTI, G. P., HSIEH, J. J. & CHENG, E. H. 2009. Stepwise activation of BAX and BAK by tBID, BIM, and PUMA initiates mitochondrial apoptosis. *Mol Cell,* 36**,** 487-99.

KLUCK, R. M., BOSSY-WETZEL, E., GREEN, D. R. & NEWMEYER, D. D. 1997a. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science,* 275**,** 1132-1136.

KLUCK, R. M., MARTIN, S. J., HOFFMAN, B., ZHOU, J. S., GREEN, D. R. & NEWMEYER, D. D. 1997b. Cytochrome c activation of CPP32-like proteolysis plays a critical role in a *Xenopus* cell-free apoptosis system. *EMBO J.,* 16**,** 4639-4649.

KOONIN, E. V., MAKAROVA, K. S., ROGOZIN, I. B., DAVIDOVIC, L., LETELLIER, M. C. & PELLEGRINI, L. 2003. The rhomboids: a nearly ubiquitous family of intramembrane serine proteases that probably evolved by multiple ancient horizontal gene transfers. *Genome Biol,* 4**,** R19.

KORSMEYER, S. J. 1992. Bcl-2 initiates a new category of oncogenes: regulators of cell death. *Blood,* 80**,** 879-886.

KOSHIBA, T., DETMER, S. A., KAISER, J. T., CHEN, H., MCCAFFERY, J. M. & CHAN, D. C. 2004. Structural basis of mitochondrial tethering by mitofusin complexes. *Science,* 305**,** 858-62.

KROEMER, G., GALLUZZI, L. & BRENNER, C. 2007. Mitochondrial membrane permeabilization in cell death. *Physiol Rev,* 87**,** 99-163.

KUWANA, T., MACKEY, M. R., PERKINS, G., ELLISMAN, M. H., LATTERICH, M., SCHNEITER, R., GREEN, D. R. & NEWMEYER, D. D. 2002. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. *Cell,* 111**,** 331-42.

LABROUSSE, A. M., ZAPPATERRA, M. D., RUBE, D. A. & VAN DER BLIEK, A. M. 1999. C. elegans dynamin-related protein DRP-1 controls severing of the mitochondrial outer membrane. *Mol Cell,* 4**,** 815-26.

LAI, Z., FERRY, K. V., DIAMOND, M. A., WEE, K. E., KIM, Y. B., MA, J., YANG, T., BENFIELD, P. A., COPELAND, R. A. & AUGER, K. R. 2001. Human mdm2 mediates multiple mono-ubiquitination of p53 by a mechanism requiring enzyme isomerization. *J Biol Chem,* 276**,** 31357-67.

LEE, Y. J., JEONG, S. Y., KARBOWSKI, M., SMITH, C. L. & YOULE, R. J. 2004. Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. *Mol Biol Cell,* 15**,** 5001-11.

LEGROS, F., LOMBES, A., FRACHON, P. & ROJO, M. 2002. Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins. *Mol Biol Cell,* 13**,** 4343-54.

LEI, X., CHEN, Y., DU, G., YU, W., WANG, X., QU, H., XIA, B., HE, H., MAO, J., ZONG, W., LIAO, X., MEHRPOUR, M., HAO, X. & CHEN, Q. 2006. Gossypol induces Bax/Bak-independent activation of apoptosis and cytochrome c release via a conformational change in Bcl-2. *FASEB J,* 20**,** 2147-9.

LEMBERG, M. K., MENENDEZ, J., MISIK, A., GARCIA, M., KOTH, C. M. & FREEMAN, M. 2005. Mechanism of intramembrane proteolysis investigated with purified rhomboid proteases. *Embo J,* 24**,** 464-72.

LETTRE, G. & HENGARTNER, M. O. 2006. Developmental apoptosis in C. elegans: a complex CEDnario. *Nat Rev Mol Cell Biol,* 7**,** 97-108.

LEU, J. I., DUMONT, P., HAFEY, M., MURPHY, M. E. & GEORGE, D. L. 2004. Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. *Nat Cell Biol,* 6**,** 443-50.

LI, H., ZHU, H. & YUAN, J. 1998. Cleavage of BID by Caspase 8 Mediates the Mitochondrial Damage in the Fas Pathway of Apoptosis. *Cell,* 94**,** 491-501.

LI, M., LUO, J., BROOKS, C. L. & GU, W. 2002. Acetylation of p53 inhibits its ubiquitination by Mdm2. *J Biol Chem,* 277**,** 50607-11.

LIU, X., KIM, C. N., YANG, J., JEMMERSON, R. & WANG, X. 1996. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell,* 86**,** 147-157.

LUO, X., BUDIHARDJO, I., ZOU, H., SLAUGHTER, C. & WANG, X. 1998. Bid, a Bcl-2 Interacting Protein, Mediates Cytochrome c release from Mitochondria in Response to Activation of Cell surface Death receptors. *Cell,* 94**,** 481-490.

LUTKENHAUS, J. 1998. Organelle division: from coli to chloroplasts. *Curr Biol,* 8**,** R619-21.

MAHYAR-ROEMER, M., FRITZSCHE, C., WAGNER, S., LAUE, M. & ROEMER, K. 2004. Mitochondrial p53 levels parallel total p53 levels independent of stress response in human colorectal carcinoma and glioblastoma cells. *Oncogene,* 23**,** 6226-36.

MAI, S., KLINKENBERG, M., AUBURGER, G., BEREITER-HAHN, J. & JENDRACH, M. Decreased expression of Drp1 and Fis1 mediates mitochondrial elongation in senescent cells and enhances resistance to oxidative stress through PINK1. *J Cell Sci,* 123**,** 917-26.

MAJEWSKI, N., NOGUEIRA, V., BHASKAR, P., COY, P. E., SKEEN, J. E., GOTTLOB, K., CHANDEL, N. S., THOMPSON, C. B., ROBEY, R. B. & HAY, N. 2004. Hexokinase-mitochondria interaction mediated by Akt is required to inhibit apoptosis in the presence or absence of Bax and Bak. *Mol Cell,* 16**,** 819-30.

MARCHENKO, N. D. & MOLL, U. M. 2007. The role of ubiquitination in the direct mitochondrial death program of p53. *Cell Cycle,* 6**,** 1718-23.

MARCHENKO, N. D., ZAIKA, A. & MOLL, U. M. 2000. Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. *J Biol Chem,* 275**,** 16202-12.

MARTINS, L. M., IACCARINO, I., TENEV, T., GSCHMEISSNER, S., TOTTY, N. F., LEMOINE, N. R., SAVOPOULOS, J., GRAY, C. W., CREASY, C. L., DINGWALL, C. & DOWNWARD, J. 2002. The serine protease Omi/HtrA2 regulates apoptosis by binding XIAP through a reaper-like motif. *J Biol Chem,* 277**,** 439-44.

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. & BOUILLET, P. 2009. The role of BH3-only protein Bim extends beyond inhibiting Bcl-2-like prosurvival proteins. *J Cell Biol,* 186**,** 355-62.

MERKWIRTH, C., DARGAZANLI, S., TATSUTA, T., GEIMER, S., LOWER, B., WUNDERLICH, F. T., VON KLEIST-RETZOW, J. C., WAISMAN, A., WESTERMANN, B. & LANGER, T. 2008. Prohibitins control cell proliferation and apoptosis by regulating OPA1-dependent cristae morphogenesis in mitochondria. *Genes Dev,* 22**,** 476-88.

MIHARA, M., ERSTER, S., ZAIKA, A., PETRENKO, O., CHITTENDEN, T., PANCOSKA, P. & MOLL, U. M. 2003. p53 has a direct apoptogenic role at the mitochondria. *Mol Cell,* 11**,** 577-90.

MISAKA, T., MURATE, M., FUJIMOTO, K. & KUBO, Y. 2006. The dynamin-related mouse mitochondrial GTPase OPA1 alters the structure of the mitochondrial inner membrane when exogenously introduced into COS-7 cells. *Neurosci Res,* 55**,** 123-33.

MIYASHITA, T. & REED, J. C. 1995. Tumor suppressor p53 is a direct transcriptional activator of the human *bax* gene. *Cell,* 80**,** 293-299.

MIZUTA, T., SHIMIZU, S., MATSUOKA, Y., NAKAGAWA, T. & TSUJIMOTO, Y. 2007. A Bax/Bak-independent mechanism of cytochrome c release. *J Biol Chem,* 282**,** 16623-30.

MOLL, U. M., LAQUAGLIA, M., BENARD, J. & RIOU, G. 1995. Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors. *Proc Natl Acad Sci U S A,* 92**,** 4407-11.

MOLL, U. M., RIOU, G. & LEVINE, A. J. 1992. Two distinct mechanisms alter p53 in breast cancer: mutation and nuclear exclusion. *Proc Natl Acad Sci U S A,* 89**,** 7262-6.

MOLL, U. M. & ZAIKA, A. 2001. Nuclear and mitochondrial apoptotic pathways of p53. *FEBS Lett,* 493**,** 65-9.

MORROW, I. C. & PARTON, R. G. 2005. Flotillins and the PHB domain protein family: rafts, worms and anaesthetics. *Traffic,* 6**,** 725-40.

MOTZ, C., MARTIN, H., KRIMMER, T. & RASSOW, J. 2002. Bcl-2 and porin follow different pathways of TOM-dependent insertion into the mitochondrial outer membrane. *J Mol Biol,* 323**,** 729-38.

MURO, I., HAY, B. A. & CLEM, R. J. 2002. The Drosophila DIAP1 protein is required to prevent accumulation of a continuously generated, processed form of the apical caspase DRONC. *J Biol Chem,* 277**,** 49644-50.

NAKAMURA, N., KIMURA, Y., TOKUDA, M., HONDA, S. & HIROSE, S. 2006. MARCH-V is a novel mitofusin 2- and Drp1-binding protein able to change mitochondrial morphology. *EMBO Rep,* 7**,** 1019-22.

NEMAJEROVA, A., ERSTER, S. & MOLL, U. M. 2005. The post-translational phosphorylation and acetylation modification profile is not the determining factor in targeting endogenous stress-induced p53 to mitochondria. *Cell Death Differ,* 12**,** 197-200.

NGUYEN, M., BRANTON, P. E., WALTON, P. A., OLTVAI, Z. N., KORSMEYER, S. J. & SHORE, G. C. 1994. Role of membrane anchor domain of Bcl-2 in suppression of apoptosis caused by E1B-defective adenovirus. *J. Biol. Chem.,* 269**,** 16521-16524.

NIJTMANS, L. G., DE JONG, L., ARTAL SANZ, M., COATES, P. J., BERDEN, J. A., BACK, J. W., MUIJSERS, A. O., VAN DER SPEK, H. & GRIVELL, L. A. 2000. Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. *Embo J,* 19**,** 2444-51.

NIKOLAEV, A. Y., LI, M., PUSKAS, N., QIN, J. & GU, W. 2003. Parc: a cytoplasmic anchor for p53. *Cell,* 112**,** 29-40.

ODA, E., OHKI, R., MURASAWA, H., NEMOTO, J., SHIBUE, T., YAMASHITA, T., TOKINO, T., TANIGUCHI, T. & TANAKA, N. 2000. Noxa, a BH3-only member of the bcl-2 family and candidate mediator of p53-induced apoptosis. *Science,* 288**,** 1053-1058.

OHTSUKA, T., RYU, H., MINAMISHIMA, Y. A., MACIP, S., SAGARA, J., NAKAYAMA, K. I., AARONSON, S. A. & LEE, S. W. 2004. ASC is a Bax adaptor and regulates the p53-Bax mitochondrial apoptosis pathway. *Nat Cell Biol,* 6**,** 121-8.

OKAMOTO, K. & SHAW, J. M. 2005. Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. *Annu Rev Genet,* 39**,** 503-36.

OLICHON, A., BARICAULT, L., GAS, N., GUILLOU, E., VALETTE, A., BELENGUER, P. & 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**,** 7743-6.

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. & BELENGUER, P. 2002. The human dynamin-related protein OPA1 is anchored to the mitochondrial inner membrane facing the inter-membrane space. *FEBS Lett,* 523**,** 171-6.

OLICHON, A., LANDES, T., ARNAUNE-PELLOQUIN, L., EMORINE, L. J., MILS, V., GUICHET, A., DELETTRE, C., HAMEL, C., AMATI-BONNEAU, P., BONNEAU, D., REYNIER, P., LENAERS, G. & BELENGUER, P. 2007. Effects of OPA1 mutations on mitochondrial morphology and apoptosis: relevance to ADOA pathogenesis. *J Cell Physiol,* 211**,** 423-30.

OLSON, M. R., HOLLEY, C. L., GAN, E. C., COLON-RAMOS, D. A., KAPLAN, B. & KORNBLUTH, S. 2003. A GH3-like domain in reaper is required for mitochondrial localization and induction of IAP degradation. *J Biol Chem,* 278**,** 44758-68. .

OTT, M., NORBERG, E., WALTER, K. M., SCHREINER, P., KEMPER, C., RAPAPORT, D., ZHIVOTOVSKY, B. & ORRENIUS, S. 2007. The mitochondrial TOM complex is required for tBid/Bax-induced cytochrome c release. *J Biol Chem,* 282**,** 27633-9.

OTT, M., NORBERG, E., ZHIVOTOVSKY, B. & ORRENIUS, S. 2009. Mitochondrial targeting of tBid/Bax: a role for the TOM complex? *Cell Death Differ,* 16**,** 1075-82.

PARK, H. H., LO, Y. C., LIN, S. C., WANG, L., YANG, J. K. & WU, H. 2007. The death domain superfamily in intracellular signaling of apoptosis and inflammation. *Annu Rev Immunol,* 25**,** 561-86.

PARONE, P. A., JAMES, D. I., DA CRUZ, S., MATTENBERGER, Y., DONZE, O., BARJA, F. & MARTINOU, J. C. 2006. Inhibiting the mitochondrial fission machinery does not prevent Bax/Bak-dependent apoptosis. *Mol Cell Biol,* 26**,** 7397-408.

PELLEGRINI, L., PASSER, B. J., CANELLES, M., LEFTEROV, I., GANJEI, J. K., FOWLKES, B. J., KOONIN, E. V. & D'ADAMIO, L. 2001. PAMP and PARL, two novel putative metalloproteases interacting with the COOH-terminus of Presenilin-1 and -2. *J Alzheimers Dis,* 3**,** 181-190.

PETIT, E., OLIVER, L. & VALLETTE, F. M. 2009. The mitochondrial outer membrane protein import machinery: a new player in apoptosis? *Front Biosci,* 14**,** 3563-70.

PETIT, P. X., LECOEUR, H., ZORN, E., DAUGUET, C., MIGNOTTE, B. & GOUGEON, M. L. 1995. Alterations in mitochondrial structure and function are early events of dexamethasone-induced thymocyte apoptosis. *J Cell Biol,* 130**,** 157-67.

PETROS, A. M., GUNASEKERA, A., XU, N., OLEJNICZAK, E. T. & FESIK, S. W. 2004. Defining the p53 DNA-binding domain/Bcl-x(L)-binding interface using NMR. *FEBS Lett,* 559**,** 171-4.

PETROS, J. A., BAUMANN, A. K., RUIZ-PESINI, E., AMIN, M. B., SUN, C. Q., HALL, J., LIM, S., ISSA, M. M., FLANDERS, W. D., HOSSEINI, S. H., MARSHALL, F. F. & WALLACE, D. C. 2005. mtDNA mutations increase tumorigenicity in prostate cancer. *Proc Natl Acad Sci U S A,* 102**,** 719-24.

PIETSCH, E. C., PERCHINIAK, E., CANUTESCU, A. A., WANG, G., DUNBRACK, R. L. & MURPHY, M. E. 2008. Oligomerization of BAK by p53 utilizes conserved residues of the p53 DNA binding domain. *J Biol Chem,* 283**,** 21294-304.

PITTS, K. R., YOON, Y., KRUEGER, E. W. & MCNIVEN, M. A. 1999. The dynamin-like protein DLP1 is essential for normal distribution and morphology of the endoplasmic reticulum and mitochondria in mammalian cells. *Mol Biol Cell,* 10**,** 4403-17.

POOLE, A. C., THOMAS, R. E., ANDREWS, L. A., MCBRIDE, H. M., WHITWORTH, A. J. & PALLANCK, L. J. 2008. The PINK1/Parkin pathway regulates mitochondrial morphology. *Proc Natl Acad Sci U S A,* 105**,** 1638-43.

QIN, H., YU, T., QING, T., LIU, Y., ZHAO, Y., CAI, J., LI, J., SONG, Z., QU, X., ZHOU, P., WU, J., DING, M. & DENG, H. 2007. Regulation of apoptosis and differentiation by p53 in human embryonic stem cells. *J Biol Chem,* 282**,** 5842-52.

QUINN, L., COOMBE, M., MILLS, K., DAISH, T., COLUSSI, P., KUMAR, S. & RICHARDSON, H. 2003. Buffy, a Drosophila Bcl-2 protein, has anti-apoptotic and cell cycle inhibitory functions. *Embo J,* 22**,** 3568-79.

ROSS, K., RUDEL, T. & KOZJAK-PAVLOVIC, V. 2009. TOM-independent complex formation of Bax and Bak in mammalian mitochondria during TNFalpha-induced apoptosis. *Cell Death Differ,* 16**,** 697-707.

SANDU, C., RYOO, H. D. & STELLER, H. 2010. Drosophila IAP antagonists form multimeric complexes to promote cell death. *The Journal of cell biology,* 190**,** 1039-52.

SCHAFER, B., QUISPE, J., CHOUDHARY, V., CHIPUK, J. E., AJERO, T. G., DU, H., SCHNEITER, R. & KUWANA, T. 2009. Mitochondrial outer membrane proteins assist Bid in Bax-mediated lipidic pore formation. *Mol Biol Cell,* 20**,** 2276-85.

SCHLEIFF, E., SHORE, G. C. & GOPING, I. S. 1997. Human mitochondrial import receptor, Tom20p. Use of glutathione to reveal specific interactions between Tom20-glutathione S-transferase and mitochondrial precursor proteins. *FEBS Lett,* 404**,** 314-8.

SCHUG, Z. T. & GOTTLIEB, E. 2009. Cardiolipin acts as a mitochondrial signalling platform to launch apoptosis. *Biochim Biophys Acta,* 1788**,** 2022-31.

SCORRANO, L., OAKES, S. A., OPFERMAN, J. T., CHENG, E. H., SORCINELLI, M. D., POZZAN, T. & KORSMEYER, S. J. 2003. BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis. *Science,* 300**,** 135-9.

SESAKI, H. & JENSEN, R. E. 2004. Ugo1p links the Fzo1p and Mgm1p GTPases for mitochondrial fusion. *J Biol Chem,* 279**,** 28298-303.

SEVRIOUKOV, E. A., BURR, J., HUANG, E. W., ASSI, H. H., MONSERRATE, J. P., PURVES, D. C., WU, J. N., SONG, E. J. & BRACHMANN, C. B. 2007. Drosophila Bcl-2 proteins participate in stress-induced apoptosis, but are not required for normal development. *Genesis,* 45**,** 184-93.

SHAHAM, S., REDDIEN, P. W., DAVIES, B. & HORVITZ, H. R. 1999. Mutational analysis of the Caenorhabditis elegans cell-death gene ced-3. *Genetics,* 153**,** 1655-71.

SHEN, Y. & SHENK, T. 1994. Relief of p53-mediated transcriptional repression by the adenovirus E1B 19-kDa protein or the cellular Bcl-2 protein. *Proc. Natl. Acad. Sci. USA,* 91**,** 8940-8944.

SHERIDAN, C., DELIVANI, P., CULLEN, S. P. & MARTIN, S. J. 2008. Bax- or Bak-induced mitochondrial fission can be uncoupled from cytochrome C release. *Mol Cell,* 31**,** 570-85.

SHIDARA, Y., YAMAGATA, K., KANAMORI, T., NAKANO, K., KWONG, J. Q., MANFREDI, G., ODA, H. & OHTA, S. 2005. Positive contribution of pathogenic mutations in the mitochondrial genome to the promotion of cancer by prevention from apoptosis. *Cancer Res,* 65**,** 1655-63.

SMIRNOVA, E., GRIPARIC, L., SHURLAND, D. L. & VAN DER BLIEK, A. M. 2001. Dynamin-related protein Drp1 is required for mitochondrial division in mammalian cells. *Mol Biol Cell,* 12**,** 2245-56.

SMIRNOVA, E., SHURLAND, D. L., RYAZANTSEV, S. N. & VAN DER BLIEK, A. M. 1998. A human dynamin-related protein controls the distribution of mitochondria. *J Cell Biol,* 143**,** 351-8.

SONG, Z., CHEN, H., FIKET, M., ALEXANDER, C. & CHAN, D. C. 2007. OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. *J Cell Biol,* 178**,** 749-55.

SOT, B., FREUND, S. M. & FERSHT, A. R. 2007. Comparative biophysical characterization of p53 with the pro-apoptotic BAK and the anti-apoptotic BCL-xL. *J Biol Chem,* 282**,** 29193-200.

SPEIDEL, D., HELMBOLD, H. & DEPPERT, W. 2006. Dissection of transcriptional and non-transcriptional p53 activities in the response to genotoxic stress. *Oncogene,* 25**,** 940-53.

STEGLICH, G., NEUPERT, W. & LANGER, T. 1999. Prohibitins regulate membrane protein degradation by the m-AAA protease in mitochondria. *Mol Cell Biol,* 19**,** 3435-42.

STENNICKE, H. R. & SALVESEN, G. S. 2000. Caspases - controlling intracellular signals by protease zymogen activation. *Biochim Biophys Acta,* 1477**,** 299-306.

STROM, E., SATHE, S., KOMAROV, P. G., CHERNOVA, O. B., PAVLOVSKA, I., SHYSHYNOVA, I., BOSYKH, D. A., BURDELYA, L. G., MACKLIS, R. M., SKALITER, R., KOMAROVA, E. A. & GUDKOV, A. V. 2006. Small-molecule inhibitor of p53 binding to mitochondria protects mice from gamma radiation. *Nat Chem Biol,* 2**,** 474-9.

SUGIOKA, R., SHIMIZU, S. & TSUJIMOTO, Y. 2004. Fzo1, a protein involved in mitochondrial fusion, inhibits apoptosis. *J Biol Chem,* 279**,** 52726-34.

SUZUKI, Y., IMAI, Y., NAKAYAMA, H., TAKAHASHI, K., TAKIO, K. & TAKAHASHI, R. 2001. A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. *Mol Cell,* 8**,** 613-21.

SYKES, S. M., STANEK, T. J., FRANK, A., MURPHY, M. E. & MCMAHON, S. B. 2009. Acetylation of the DNA binding domain regulates transcription-independent apoptosis by p53. *J Biol Chem,* 284**,** 20197-205.

TAGUCHI, N., ISHIHARA, N., JOFUKU, A., OKA, T. & MIHARA, K. 2007. Mitotic phosphorylation of dynamin-related GTPase Drp1 participates in mitochondrial fission. *J Biol Chem,* 282**,** 11521-9.

TAN, J., ZHUANG, L., LEONG, H. S., IYER, N. G., LIU, E. T. & YU, Q. 2005. Pharmacologic modulation of glycogen synthase kinase-3beta promotes p53-dependent apoptosis through a

direct Bax-mediated mitochondrial pathway in colorectal cancer cells. *Cancer Res,* 65**,** 9012-20.

TANG, S., LE, P. K., TSE, S., WALLACE, D. C. & HUANG, T. 2009. Heterozygous mutation of Opa1 in Drosophila shortens lifespan mediated through increased reactive oxygen species production. *PLoS One,* 4**,** e4492.

TANG, X., ZHU, Y., HAN, L., KIM, A. L., KOPELOVICH, L., BICKERS, D. R. & ATHAR, M. 2007. CP-31398 restores mutant p53 tumor suppressor function and inhibits UVB-induced skin carcinogenesis in mice. *J Clin Invest,* 117**,** 3753-64.

TOMITA, Y., MARCHENKO, N., ERSTER, S., NEMAJEROVA, A., DEHNER, A., KLEIN, C., PAN, H., KESSLER, H., PANCOSKA, P. & MOLL, U. M. 2006. WT p53, but not tumor-derived mutants, bind to Bcl2 via the DNA binding domain and induce mitochondrial permeabilization. *J Biol Chem,* 281**,** 8600-6.

TONDERA, D., GRANDEMANGE, S., JOURDAIN, A., KARBOWSKI, M., MATTENBERGER, Y., HERZIG, S., DA CRUZ, S., CLERC, P., RASCHKE, I., MERKWIRTH, C., EHSES, S., KRAUSE, F., CHAN, D. C., ALEXANDER, C., BAUER, C., YOULE, R., LANGER, T. & MARTINOU, J. C. 2009. SLP-2 is required for stress-induced mitochondrial hyperfusion. *Embo J,* 28**,** 1589-600.

VAN LOO, G., VAN GURP, M., DEPUYDT, B., SRINIVASULA, S. M., RODRIGUEZ, I., ALNEMRI, E. S., GEVAERT, K., VANDEKERCKHOVE, J., DECLERCQ, W. & VANDENABEELE, P. 2002. The serine protease Omi/HtrA2 is released from mitochondria during apoptosis. Omi interacts with caspase-inhibitor XIAP and induces enhanced caspase activity. *Cell Death Differ,* 9**,** 20-6.

VAYSSIERE, J. L., PETIT, P. X., RISLER, Y. & MIGNOTTE, B. 1994. Commitment to apoptosis is associated with changes in mitochondrial biogenesis and activity in cell lines conditionally immortalized with simian virus 40. *Proc Natl Acad Sci U S A,* 91**,** 11752-6.

VERHAGEN, A. M., EKERT, P. G., PAKUSCH, M., SILKE, J., CONNOLLY, L. M., REID, G. E., MORITZ, R. L., SIMPSON, R. J. & VAUX, D. L. 2000. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell,* 102**,** 43-53.

VOUSDEN, K. H. & LANE, D. P. 2007. p53 in health and disease. *Nat Rev Mol Cell Biol,* 8**,** 275-83.

WAKABAYASHI, J., ZHANG, Z., WAKABAYASHI, N., TAMURA, Y., FUKAYA, M., KENSLER, T. W., IIJIMA, M. & SESAKI, H. 2009. The dynamin-related GTPase Drp1 is required for embryonic and brain development in mice. *J Cell Biol,* 186**,** 805-16.

WANG, C. & YOULE, R. J. 2009. The Role of Mitochondria in Apoptosis. *Annu Rev Genet,* 43**,** 95-118.

WASILEWSKI, M. & SCORRANO, L. 2009. The changing shape of mitochondrial apoptosis. *Trends Endocrinol Metab,* 20**,** 287-94.

WESTERMANN, B. 2008. Molecular machinery of mitochondrial fusion and fission. *J Biol Chem,* 283**,** 13501-5.

WHITE, K., GRETHER, M. E., ABRAMS, J. M., YOUNG, L., FARRELL, K. & STELLER, H. 1994. Genetic control of programmed cell death in *Drosophila*. *Science,* 264**,** 677-683.

WILSON, R., GOYAL, L., DITZEL, M., ZACHARIOU, A., BAKER, D. A., AGAPITE, J., STELLER, H. & MEIER, P. 2002. The DIAP1 RING finger mediates ubiquitination of Dronc and is indispensable for regulating apoptosis. *Nat. Cell Biol.,* 4**,** 445-450.

WOLOZIN, B., IWASAKI, K., VITO, P., GANJEI, J. K., LACANA, E., SUNDERLAND, T., ZHAO, B., KUSIAK, J. W., WASCO, W. & D'ADAMIO, L. 1996. Participation of presenilin 2 in apoptosis: enhanced basal activity conferred by an Alzheimer mutation. *Science,* 274**,** 1710-3.

XU, D., LI, Y., ARCARO, M., LACKEY, M. & BERGMANN, A. 2005. The CARD-carrying caspase Dronc is essential for most, but not all, developmental cell death in Drosophila. *Development,* 132**,** 2125-34.

YAMAGUCHI, R., LARTIGUE, L., PERKINS, G., SCOTT, R. T., DIXIT, A., KUSHNAREVA, Y., KUWANA, T., ELLISMAN, M. H. & NEWMEYER, D. D. 2008. Opa1-mediated cristae opening is Bax/Bak and BH3 dependent, required for apoptosis, and independent of Bak oligomerization. *Mol Cell,* 31**,** 557-69.

YANG, J., LIU, X., BHALLA, K., KIM, C. N., IBRADO, A. M., CAI, J., PENG, T. I., JONES, D. P. & WANG, X. 1997. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science,* 275**,** 1129-1132.

YIN, X. M. 2006. Bid, a BH3-only multi-functional molecule, is at the cross road of life and death. *Gene,* 369**,** 7-19.

YOO, S. J., HUH, J. R., MURO, I., YU, H., WANG, L., WANG, S. L., FELDMAN, R. M., CLEM, R. J., MULLER, H. A. & HAY, B. A. 2002. Hid, Rpr and Grim negatively regulate DIAP1 levels through distinct mechanisms. *Nat Cell Biol,* 4**,** 416-24.

YOON, Y., KRUEGER, E. W., OSWALD, B. J. & MCNIVEN, M. A. 2003. The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamin-like protein DLP1. *Mol Cell Biol,* 23**,** 5409-20.

YOON, Y. & MCNIVEN, M. A. 2001. Mitochondrial division: New partners in membrane pinching. *Curr Biol,* 11**,** R67-70.

YOSHIDA, Y., IZUMI, H., TORIGOE, T., ISHIGUCHI, H., ITOH, H., KANG, D. & KOHNO, K. 2003. P53 physically interacts with mitochondrial transcription factor A and differentially regulates binding to damaged DNA. *Cancer Res,* 63**,** 3729-34.

YOULE, R. J. & STRASSER, A. 2008. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol,* 9**,** 47-59.

ZAMZAMI, N., MARCHETTI, P., CASTEDO, M., ZANIN, C., VAYSSIÈRE, J. L., PETIT, P. X. & KROEMER, G. 1995. Reduction in mitochondrial potential constitutes an early irreversible step of programmed lymphocyte death *in vivo*. *J. Exp. Med.,* 181**,** 1661-1672.

ZANNA, C., GHELLI, A., PORCELLI, A. M., KARBOWSKI, M., YOULE, R. J., SCHIMPF, S., WISSINGER, B., PINTI, M., COSSARIZZA, A., VIDONI, S., VALENTINO, M. L., RUGOLO, M. & CARELLI, V. 2008. OPA1 mutations associated with dominant optic atrophy impair oxidative phosphorylation and mitochondrial fusion. *Brain,* 131**,** 352-67.

ZHANG, H., KIM, J. K., EDWARDS, C. A., XU, Z., TAICHMAN, R. & WANG, C. Y. 2005. Clusterin inhibits apoptosis by interacting with activated Bax. *Nat Cell Biol,* 7**,** 909-15.

ZHONG, L. T., SARAFIAN, T., KANE, D. J., CHARLES, A. C., MAH, S. P., EDWARDS, R. H. & BREDESEN, D. E. 1993. *bcl-2* inhibits death of central neural cells induced by multiple agents. *Proc. Natl. Acad. Sci. USA,* 90**,** 4533-4537.

ZHU, W., COWIE, A., WASFY, G. W., PENN, L. Z., LEBER, B. & ANDREWS, D. W. 1996. Bcl-2 mutants with restricted subcellular location reveal spatially distinct pathways for apoptosis in different cell types. *EMBO J.,* 15**,** 4130-4141.

		Nematode	***Drosophila***	**Mammals**
Caspases	Initiator caspases	CED-3	DRONC	Casp-9, -8…
	Effector caspases	CED-3	DrIce, DREDD …	Casp-3, -7…
	Adaptators	CED-4	Dark	Apaf-1
Bcl-2 family	Antiapoptotic proteins	CED-9	Buffy	Bcl-2, Bcl-x_L...
	Proapoptotic proteins		Debcl	Bax, Bak, Bok
	Proapoptotic BH3 only proteins (BOP)	EGL-1	?	BID, BIM, PUMA…
	Inhibitors of caspases	CSP-3	DIAP1, DIAP2…	c-IAP1, c-IAP2…
	Disruptors of the anti-caspase activity of IAPs		RPR, HID, GRIM, SICKLE	Smac/Diablo, Omi/HtrA2

Table I. Conservation during evolution of proteins involved in the mitochondrial pathway of caspases activation.

Three families of proteins involved in apoptosis are found in nematodes, *Drosophila* and mammals: CED-3, -4, -9 are homologous to caspases, caspase activators (such as Apoptosis activating factor 1, Apaf-1) and proteins of the Bcl-2 (B Cell Lymphoma 2) family. In *Drosophila* and mammals, caspase inhibitors (IAPs, Inhibitor of Apoptosis Proteins) and proteins able to abrogate this inhibition also participate in the control of caspase activity (Smac/Diablo, RPR, HID, GRIM…). Note that in *C. elegans* CED-3 can be inhibited by CSP-3, a partial caspase homologue unrelated to IAPs, to prevent CED-3 auto-activation (review : (Brady and Duckett, 2009))

Figure legends

Figure 1. Main regulators of the mitochondrial apoptotic pathway in *C. elegans*, *Drosophila* and mammals. In all these species, this apoptotic pathway relies on caspase activation into an apoptosome. The apoptosome contains at least an oligomer of caspase activator (CED-4, Dapaf-1 or Apaf-1) and several copies of a CARD-carrying initiator caspase (CED-3, DRONC or Caspase-9). In mammals, cytochrome c is an additional co-factor required for apoptosome formation. In these three clades, Bcl-2 family members, which are either proapoptotic (EGL-1, Debcl, BOP or Bax) or antiapoptotic (CED-9, Buffy, Bcl-2) regulate each other at the level of the mitochondria and are involved in the regulation of apoptosome activation. Nonetheless, their mode of action differs: in the nematode, the antiapoptotic protein CED-9 prevents apoptosome formation by direct binding to CED-4, and EGL-1 promotes apoptosome formation by releasing CED-4 from CED-9. In *Drosophila*, Bcl-2 proteins are localized to mitochondria and the proapoptotic Debcl induces an apoptosome-dependent cell death, in which the role of cytochrome c remains unclear. In mammals, proteins of the Bcl-2 family either promote (such as Bax or BH3-only proteins) or inhibit (such as Bcl-2) the release of apoptogenic factors from the mitochondrial intermembrane space to the cytosol, one of which being the Apaf-1 co-factor, cytochrome c. In both mammals and *Drosophila*, apoptosome activity can be limited by IAPs.

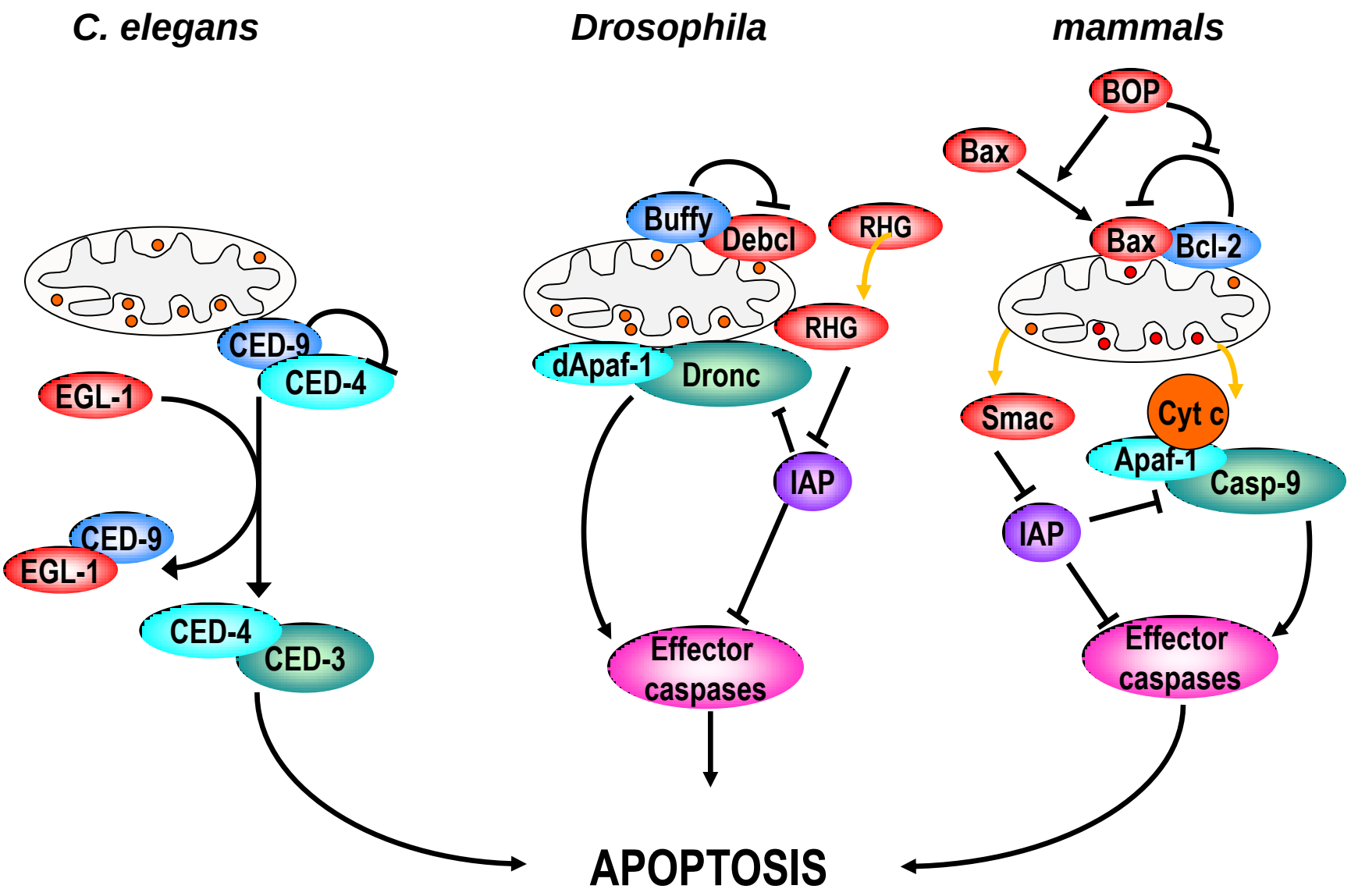
Figure 2: Interaction between BH3 only proteins and survival members of the Bcl-2 family. A class of BOP (*i.e.* BIM, PUMA and BID) binds to all survival Bcl-2 like proteins (and most likely to the proapoptotic ones) while other BOPs only bind to a subset of these proteins. Apoptosis can only be triggered when selective interactions occur following activation of specific signals. A complete database on the BCL-2 family can be found at http://bcl2db.ibcp.fr/

Figure 3: Two models of activation of BAX and BAK.

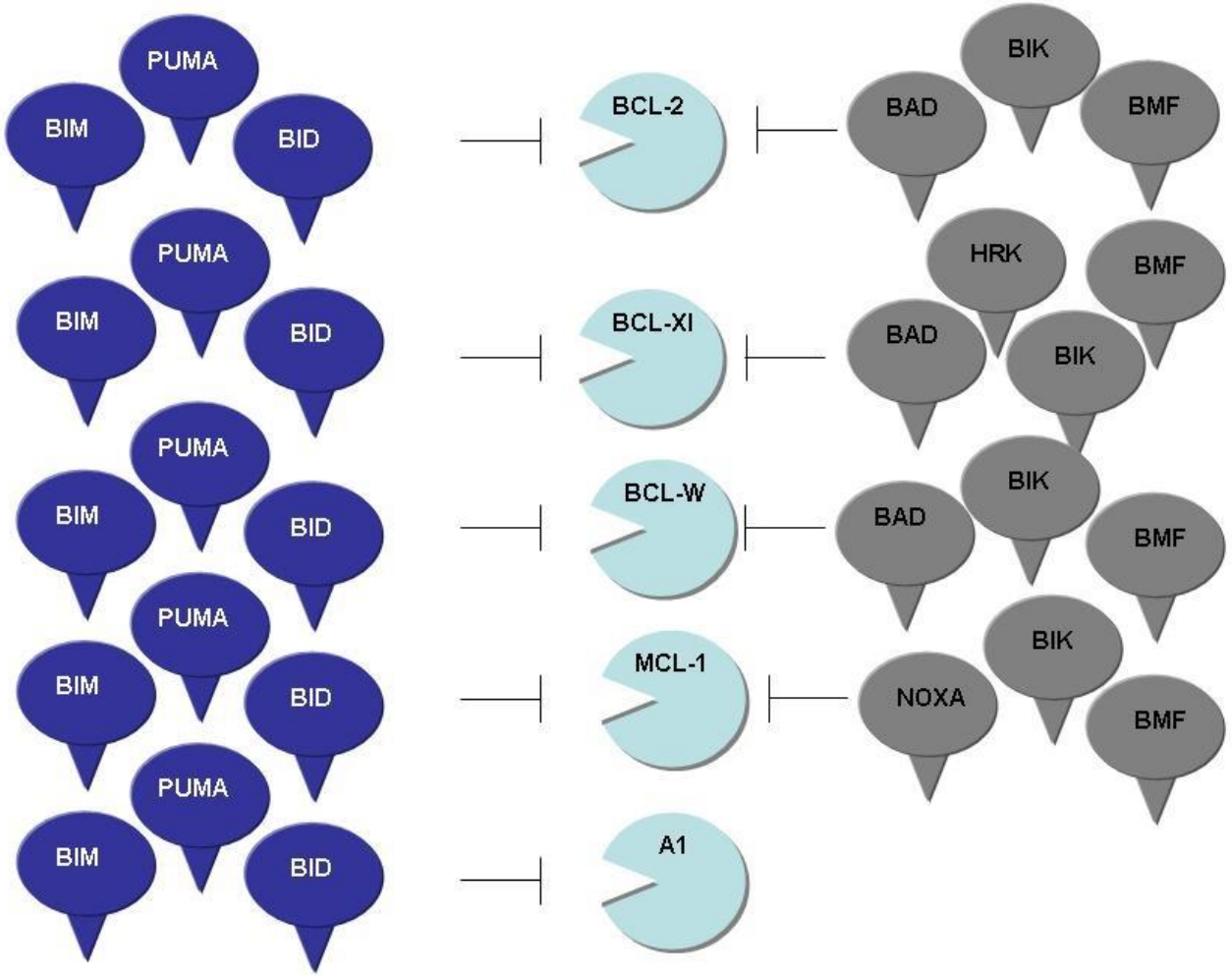
A) For Bax activation, the primary event in the activation of apoptosis is a change of conformation induced by the transient interaction with BID (or PUMA or BIM) which leads to a change of conformation that facilitates the insertion of Bax into mitochondria. The inhibition of Bax by Bcl-2 or other prosurvival proteins of the BCL-2 family is prevented by a competitive inhibition with BOPs with the specificity depicted in figure 1. Bak activation is roughly similar to Bax's.

B) In this model, BOPs intervention is divided into two steps: firstly, BOPs liberate PUMA/BID/ BIM complexed to prosurvival proteins and secondly, the liberated PUMA/BID/ BIM are thus able to activate Bax or Bak.

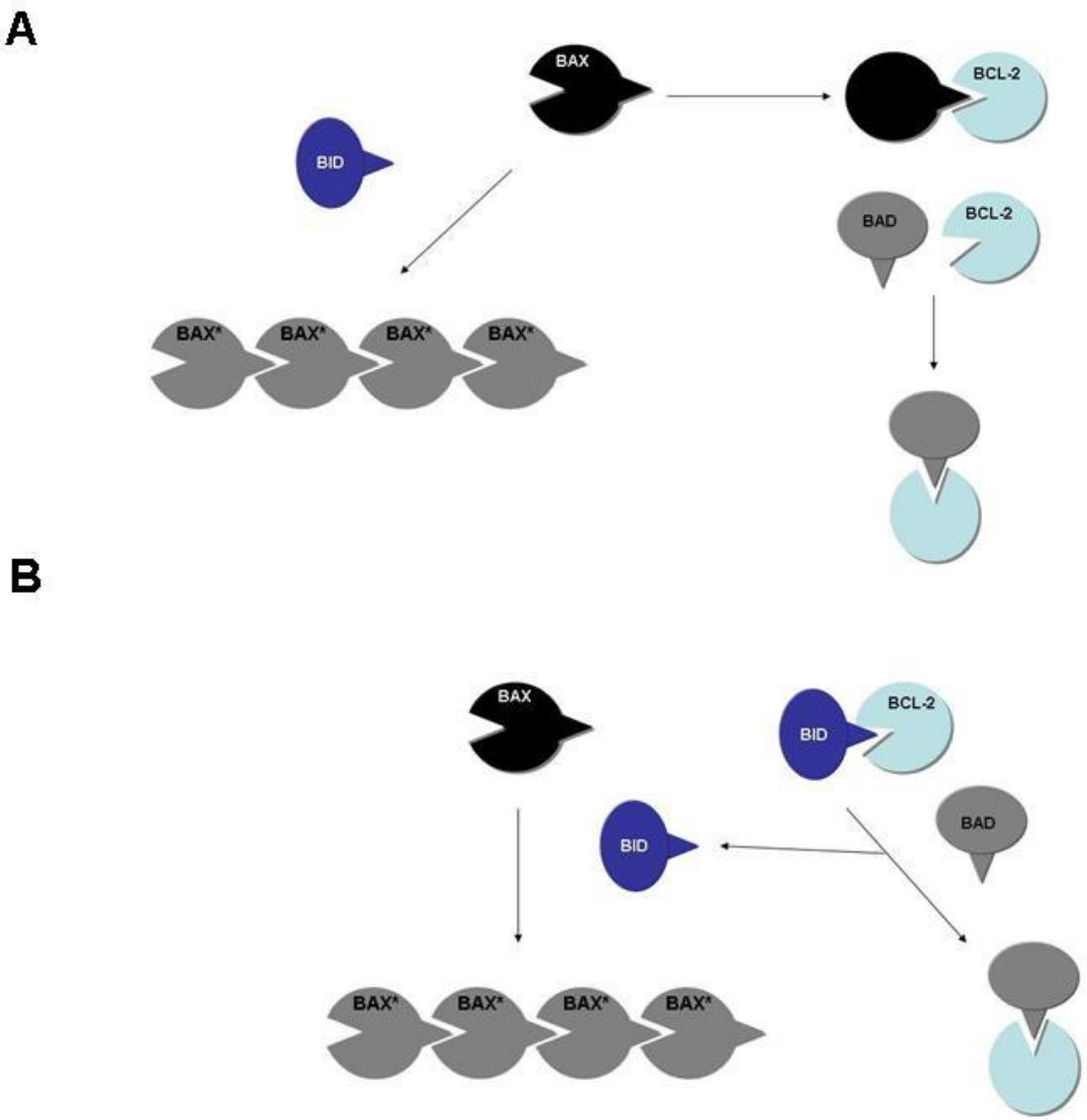
Abbreviations: ANT: adenine nucleotide translocase, BOP: BH3 only protein; CARD: caspase activation and recruitment domain; DED: death effector domain; Dronc: *Drosophila* nedd-2 like caspase; CED: cell death; EGL-1:egg-laying-1; Bcl-2: B-cell lymphoma 2; caspase: cysteine aspartase, IAP: inhibitor of apoptosis protein; BH: Bcl-2 homology, MOMP: mitochondrial outer membrane permeabilization, PTP: permeability transition pore, TOM: translocase of the outer membrane, VDAC: voltage-dependent anion channel



Chapter 8 - Figure 1



Chapter 8 - Figure 2



Chapter 8 - Figure 3