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**Abstract**

Mitochondria evolved as an endosymbiont providing the cell with a dizzying array of catabolic and anabolic processes essential for life. However, mitochondria have retained the ability to kill from within, and are widely considered the final executioners of programmed cell death. The groundbreaking discovery over 25 years ago that mitochondrial cytochrome c is released into the cytosol shone new and unexpected light onto this old organelle, revitalizing the field. The Bcl-2 family of proteins plays a central role in the maintenance of mitochondrial membrane integrity, but other factors are also involved in the cell death program. Indeed, contacts with the endoplasmic reticulum (ER), mitochondrial division and inner membrane cristae remodeling have emerged as key regulators of cytochrome c release. This review will focus on recent progress to define the functional contribution of the apoptotic ER/ mitochondrial interface, which couples mitochondrial fission and cristae remodeling to calcium and lipid fluxes.


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**The mitochondria-Endoplasmic Reticulum contact sites: A signalling platform for Cell Death**

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-3 Figures
-Papers of special interest (•) or outstanding interest (••) indicated, with short commentaries.

## HIGHLIGHTS

- Drp1-SUMOylation stabilizes the mitochondrial/ER interface required for apoptosis
- This mitochondrial/ER platform facilitates calcium and lipid fluxes during cell death
- Drp1 and mitochondrial fragmentation are crucial for cristae remodeling during apoptosis
- OPA1-oligomer cleavage and cristae remodeling are $Ca^{2+}$-dependent

## ABSTRACT (153 CHARACTERS)

Mitochondria evolved as an endosymbiont providing the cell with a dizzying array of catabolic and anabolic processes essential for life. However, mitochondria have retained the ability to kill from within, and are widely considered the final executioners of programmed cell death. The groundbreaking discovery over 25 years ago that mitochondrial cytochrome c is released into the cytosol shone new and unexpected light onto this old organelle, revitalizing the field. The Bcl-2 family of proteins plays a central role in the maintenance of mitochondrial membrane integrity, but other factors are also involved in the cell death program. Indeed, contacts with the endoplasmic reticulum (ER), mitochondrial division and inner membrane cristae remodeling have emerged as key regulators of cytochrome c release. This review will focus on recent progress to define the functional contribution of the apoptotic ER/mitochondrial interface, which couples mitochondrial fission and cristae remodeling to calcium and lipid fluxes.

## INTRODUCTION

Mitochondria are essential organelles responsible for an array of biochemical reactions critical for survival and homeostatic adaptation of the cell. These broad metabolic functions are tightly linked to mitochondrial architecture, or shape [1]. Mitochondria are highly dynamic, altering their shape in response to various cellular cues. These responses include changes in mitochondrial fusion, division, cristae remodeling and motility within the cell. The challenge has been in determining the functional contribution of these dynamic changes in architecture to both signalling and metabolic programs.

One important element of mitochondrial dynamics is the modulation of contact sites with other organelles, particularly for the acquisition of metabolites that lie at the heart of mitochondrial function [2,3]. The best characterized of these dynamic contacts are those between the mitochondria and the endoplasmic reticulum (ER), commonly referred to as Mitochondria-Associated Membrane (MAM), representing almost 20% of the mitochondrial surface [4]. However, mitochondrial contacts with other organelles including early and late endosomes, lipid droplets and peroxisomes also play important roles in the exchange of metabolites. The molecular mechanisms that regulate mitochondrial contacts appear to be cell and context dependent [5]. For example, ER contacts rapidly adapt to the metabolic status of the cell [6], and can be stabilized during cell death [7••].

There are a number of tethering complexes and mechanisms that co-ordinate ER/mitochondrial contacts, including the machinery that regulates mitochondrial fission and fusion [5]. First, the fusion GTPase Mitofusin2 (Mfn2) tethers mitochondria to the ER to facilitate the flux of calcium between these organelles [8-10]. In addition to the links to the fusion GTPase Mfn2, ER contacts were also seen at sites of mitochondrial division, coupling the activity of the fission GTPase Dynamin related protein 1 (Drp1) to ER tethering [11]. Mechanistically it is unclear how the ER identifies and marks the specific sites for division, but it is likely to be tightly regulated through molecular tethers and signalling machinery. Indeed, some of the fission-related ER contacts occur at sites of mtDNA replication, hinting that signals from the replicating mtDNA nucleoids activate the formation of these contacts [12,13••]. Together these findings helped to establish that mitochondrial morphology transitions are intimately coupled to ER contact sites. While there are many functions for ER/mitochondrial contacts [14,15], we focus here on the functional contribution of fission-related contact sites in the process of apoptosis.

## Architectural transitions in mitochondria drive apoptosis.

The mitochondrial pathway of apoptosis is a natural process contributing to cell homeostasis and is regulated by signalling through the Bcl-2 family of proteins [16]. Dysregulation of this process has been studied extensively as a driver of numerous diseases, particularly in cancer, where apoptosis is limited [17], and degenerative diseases [18] where excessive cell death predominates. Ultimately the antagonism between pro- and the anti-apoptotic proteins of this family control the permeabilization of the Outer Mitochondrial Membrane (OMM), allowing the release of cytochrome c and other resident proteins of the InterMembrane Space (IMS) [19]. Indeed, after activation by BH3 only proteins, the pro-apoptotic members BAX and BAK oligomerize at the OMM to form an expanding pore [20•,21•]. The release of cytochrome c is considered a "point of no return" since it contributes to the formation of the apoptosome, activating signaling pathways, protease cascades and subsequent cell death [22] (**Figure 1**). In healthy cells, cytochrome c is an essential component of the electron transport chain, transferring electrons between Complex III and IV. It is localized within the IMS where it binds tightly to cardiolipin on the outer leaflet of the Inner Mitochondrial Membrane (IMM), and is mainly locked inside the mitochondrial cristae [23] (**Figure 1B, C**). Cristae are mitochondrial structures connected to the boundary IMS narrow tubular junctions, which are controlled by the MICOS complex and the profusion GTPase OPA1 [24,25]. During cell death, oligomers of membrane-bound

form of OPA1 are disrupted, leading to the remodeling and opening of the cristae junctions, allowing cytochrome c release [26] (**Figure 1C, D**). Indeed, in healthy cells, there is a constant balance between the different isoforms of OPA1, the membrane-anchored form of OPA1 (L-OPA1) and the soluble and shorter fragments (S-OPA1). During cell death, the oligomeric L-OPA1 forms are cleaved to generate the S-OPA1 isoforms, which contribute to the remodeling of the IMM [27] (**Figure 1C, D**). In contrast, maintenance of OPA1 oligomers is protective against cell death [28] and transgenic mice overexpressing OPA1 were protected within models of neurodegeneration and cardiac hypertrophy [29•,30].

While the dynamics of the inner membrane are central to the death program, it is also clear that Drp1-dependent mitochondrial fragmentation is coupled to apoptosis [31-34]. However, it has been less clear whether or why the overall size of the organelle would matter for the execution of cell death [35-38•]. We submit that the size may not matter in the end, rather the stabilization of fission-related ER contact sites facilitates the assembly of apoptotic signalling complexes that regulate lipid and calcium flux into the mitochondria.

## Apoptosis is coupled to mitochondrial remodeling.

Drp1 is a cytosolic protein, which is recruited to the OMM through specific receptors to drive mitochondrial division in steady state. There are a number of mitochondrial receptors for Drp1, including the Mitochondrial Fission Factor (MFF) [39,40], and the Mitochondrial Dynamics proteins of 49 kDa (MiD49) and 51 kDa (MiD51) [41-44]. In order to understand why mitochondria fragment during cell death, it is important to understand exactly when it occurs. Most studies are consistent with a model whereby Drp1 is recruited by its receptors following the activation and mitochondrial targeting of BAX/BAK, but before the release of cytochrome c [34,45,46] (**Figure 1**). The requirement for Drp1 and its receptors in cell death was confirmed using genetic ablation in multiple systems [40,41,47]. Early studies revealed that the absence of Drp1 and mitochondrial fission led to a block in the remodeling of the cristae, providing at least a partial explanation as to why cytochrome c release was delayed [48]. However the mechanisms that coupled Drp1 action to cristae remodeling remained elusive [48]. In addition, a recent study identified a role for the canonical Dynamin 2 in the final steps of mitochondrial division, where loss of Dynamin 2 led to a delay in cytochrome c release during cell death [49••]. Consistent with a requirement for mitochondrial fragmentation in apoptosis, activation of mitochondrial fusion through either Mfn1 or Mfn2 protects against cell death [38•,50-53]. Overall, ~15 years of research has established a role for mitochondrial fragmentation – and inactivation of fusion -

downstream of BAX/BAK activation, prior to cytochrome c release. However, it is important to note that some studies have uncoupled mitochondrial fragmentation from cytochrome c release, indicating that Drp1 and division may be dispensable in some death paradigms [35-37,54,55].

## ER/mitochondria contact sites drive the apoptotic fission process

### MAPL SUMOylates Drp1 to stabilize apoptotic ER/mitochondria contact sites

Apoptotic fission is distinct from steady state fission in that following BAX/BAK activation, Drp1 no longer cycled on and off the membrane, rather it becomes trapped and stabilized at the site of fission [56]. This coincided with Drp1 SUMOylation at sites of mitochondrial constriction and fission. However, it was unclear how SUMOylation was regulated during cell death, nor was it shown whether SUMOylation was requisite for cell death. Answers to these questions came from the identification of the Mitochondrial Anchored Protein Ligase (MAPL/MUL1), a SUMO E3 ligase [57]; along with the realization that fission occurs at ER contact sites [11-13••,58••] (**Figure 1**). MAPL is a peroxisomal and mitochondrial protein, stably inserted in the OMM via 2 transmembrane domains with the C- and N-terminus facing the cytosol and a large ~40kDa IMS domain [59]. The ligase activity is ensured by a RING domain of MAPL at the C-terminus [57], and loss of MAPL led to an inhibition of cell death [7••]. MAPL appears to have a dual function as both a ubiquitin and SUMO E3 ligase [7••,57,60-64]. Interestingly, at least one other RING finger type E3 ligase, TOPORS, can both ubiquitinate and SUMOylate targets, depending on the situation [65-69]. TOPORS and MAPL are on the same phylogenetic branch within the RING finger family of proteins, perhaps consistent with a shared dual ligase activity [70]. It is possible that these ligases are capable of forming mixed SUMO/ubiquitin chains via internal conjugation sites [71,72]. Alternatively, the specificity for ubiquitin or SUMO may be dependent upon the activating trigger.

Adding to this complexity, SUMO proteases can distinguish between the SUMO isoforms SUMO1, SUMO2, SUMO3 and SUMO4, and between the linkages within SUMO chains [73-76]. Indeed, a number of studies examined the contribution of SenP2, SenP3 and SenP5 to events at the mitochondria, with varying results. These studies have shown that deSUMOylation may inhibit cell death [74,77•,78], or promote it [79,80]. Whether and how SUMO proteases may edit mixed chains, and act in response to context and cell specific death programs remains to be clarified.

Consistent with the role for MAPL in SUMOylating Drp1 to promote mitochondrial division, immunofluorescence of HeLa cells overexpressing FLAG-MAPL showed that FLAG-MAPL accumulated at mitochondria/ER contact sites that marked scission events [7••]. During a death trigger, Drp1 was specifically SUMOylated by MAPL at ER contact sites, where YFP-SUMO1 was seen to accumulate. Upon loss of MAPL, YFP-SUMO1 no longer accumulated at sites of constriction, and cytochrome c release was delayed, confirming MAPL as a requisite SUMO E3 ligase during apoptosis [7••]. Consistent with the requirement for SUMOylation in cell death, the ectopic mitochondrial targeting of the SUMO protease SenP5 fully phenocopied the loss of MAPL, providing additional evidence that the apoptotic fission site is modulated by the SUMOylation (rather than ubiquitination) activity of MAPL [7••,81,82].

Importantly, the loss of SUMO1 conjugation also led to a reduction in ER contact sites both in steady state and during cell death, resulting in a functional decrease of calcium uptake into mitochondria during apoptosis [7••]. Indeed, one described function of the mitochondria/ER interface is the calcium signaling between the 2 organelles, where calcium released from the ER via the inositol 1,4,5-trisphosphate receptors (IP3R) will be transported into the mitochondria [83] through the Voltage-Dependent Anion Channel (VDAC) at the OMM [84] and the Mitochondrial Calcium Uniporter (MCU) in the IMM [85] (**Figure 1**). Consistent with a decrease in calcium uptake, mitochondrial ultrastructure revealed that loss of MAPL or Drp1 resulted in an delay in OPA1-oligomer disassembly and cristae remodeling [7••], a process previously shown to be dependent on calcium uptake from the ER [48] (**Figure 1**).

### Apoptotic ER/mitochondria contact sites are different from healthy contact sites

The apoptotic mitochondria/ER constriction site shows unique features, consistent with these sites having additional and distinct functions. Indeed, MAPL is not required for steady state mitochondrial fission, as its loss does not fully phenocopy the loss of Drp1 [7••]. This indicates that the rapid ~30-60 second fission events in steady state do not require SUMOylation for Drp1 conformational transitions. On the other hand, overexpression of MAPL promotes mitochondrial fragmentation; so while not essential, MAPL has a strong capacity to promote mitochondrial fission [57,59]. Given the block in Drp1 recycling on and off the mitochondria during cell death [56], it is likely that SUMOylation of the oligomeric ring may inhibit GTP hydrolysis and constriction of the

oligomer [7••,56]. Instead, the oligomer remains stable, promoting a platform to facilitate metabolic flux between the ER and mitochondria. Therefore, the steady state function of MAPL may be to stabilize specific mitochondria/ER constriction sites, kinetically delaying the division event in order to facilitate metabolic flux in healthy cells. These stable contacts would ultimately resolve in fission, providing an explanation for the robust pro-fission activity of overexpressed MAPL.

### Regulation of death-induced mitochondrial SUMOylation

SUMO and ubiquitin E3 ligases generally have many substrates, however Drp1 appears to be the main target that stabilizes the apoptotic fission site. In death-activated cells, fractionation of mitochondria revealed a dramatic increase in many SUMOylated proteins, which was tightly dependent on MAPL and Drp1[7••]. These data suggest that the stabilized Drp1 oligomer may act as a seed for the SUMOylation of other components within the fission complex. These could also include components of the ER/mitochondrial contact sites that drive actin polymerization at sites of membrane constriction, like the Inverted Formin 2 (INF2) and the mitochondrial SPIR1C protein, which could anchor the fission-specific contact site [86-89]. However, the identities of the MAPL-dependent SUMO targets that accumulate during cell death are currently unknown, and future work will provide important insights into the global function of SUMOylation within the apoptotic paradigm.

Since SUMOylation is activated during cell death, what regulates the enzymatic activity of MAPL? To date, the data indicate that Drp1 SUMOylation is critically dependent on BAX/BAK activation [56], suggesting that BAX/BAK recruitment to the mitochondrial membrane is a core activator of MAPL. Drp1 is further regulated through phosphorylation events, where protein kinase A (PKA) phosphorylation at S637 inhibits mitochondrial division (**Figure 2**). The mitochondrial PKA anchoring protein AKAP1 is degraded during cell death [90], and the phosphatase calcineurin becomes activated, which together promote dephosphorylation at this site [91-93]. In cells lacking MAPL, AKAP1 was still degraded, and Drp1 was dephosphorylated at S637, indicating that SUMOylation occurs downstream of the Drp1 kinase/phosphatase transition [7••] (**Figure 2**). Whether MAPL activity is regulated through direct, BAX/BAK dependent post-translational modifications, or whether the phosphorylation and assembly states of Drp1 determine substrate specificity remains to be determined.

## The consequences of stabilized ER/mitochondrial contact sites in cell death.

Ultimately, stabilized ER/mitochondrial contacts allow prolonged calcium flux into mitochondria, thereby activating the permeability transition pore, driving mitochondrial depolarization [94], and cristae remodeling [7••,48,95••] (**Figure 1**). This is consistent with the long established role of calcium flux at the ER/mitochondria interface in cell death [83,96•-101]. Interestingly, functional links between tumor suppressors and the establishment of ER/mitochondrial contacts have also been highlighted in different cancer cell models. For example, it has been shown that the tumor suppressors P53, PML and PTEN localize to ER/mitochondrial contact sites, and were required for apoptosis [102-104]. Taking together, these data indicate the relevance and complexity of the mitochondria/ER platform in cell death, and the importance of these contacts in regulating cancer progression.

While clearly important, the precise molecular events that couple calcium flux to cristae remodeling have not yet been established. A major regulator of cristae morphology is the inner membrane GTPase OPA1, a protein that is cleaved during cell death [26,34,105-109]. Yet the major proteases responsible for OPA1 cleavage have not been linked to calcium [27]. However, $Ca^{2+}$-dependent proteases, the calpains, are localized to mitochondria and may be involved in the regulation of OPA1 and mitochondrial morphology. Indeed, Calpastatin, an endogenous calpain inhibitor, rescued mitochondrial morphology and cell death induced by excitotoxicity in neurons in OPA1-dependent manner [110]. Therefore calcium, or perhaps the co-ordinate regulation of local ROS flux [95••], may activate different proteases that cleave the main regulator of cristae junction assembly, OPA1 [27]. Interestingly, the zinc metalloprotease OMA1, known to cleave OPA1 into the short form [108,109], has recently been highlighted in OPA1-dependent cristae remodeling in U2OS cells during cell death, however calcium dependence of this event was not tested [111••]. Interestingly, it has been previously demonstrated that a death-induced transient and rapid loss of the mitochondrial electrochemical potential was coupled to calcium (or perhaps ROS) influx to the matrix [95••,112,113]. A loss in potential is known to activate OMA1 [108,109], providing a potential link between calcium uptake and OPA1 disassembly.

## Lipid exchange at the apoptotic ER/mitochondria interface

While stabilized ER contacts facilitate calcium flux important to drive cristae remodeling and cytochrome c release, it is likely that these contacts also contribute to lipid exchange. This lipid flux may participate to the transitions in membrane architecture and/or

contribute to the assembly of BAX/BAK channel assembly (**Figure 3**). The stabilization of highly curved membranes driven by DRP1-dependent mitochondrial constriction sites was shown to facilitate BAX oligomerization, providing a biophysical explanation for the increased efficiency of cell death when constriction sites are stabilized [114]. In addition, ceramides accumulate in mitochondria during cell death, and there is convincing evidence demonstrating a contribution to BAX channel assembly [115-119]. Ceramides are generated within the ER through the sphingomyelin pathway, enzymes enriched in the MAM (**Figure 3**). A recent study revealed the protective role for a MAM-enriched, Bcl-2 related protein called Bcl-2-L13 (also called Bcl-rambo) in the direct inhibition of ceramides synthesis, thereby blocking cell death [120•,121]. In contrast, the pro-apoptotic protein BAK was shown to bind and activate ceramide synthase [117], further highlighting the complex regulation of ceramide production during the death program (**Figure 3**). In addition, the metabolites derived from ceramide, sphingosine-1-phosphate and hexedecanal were also shown to promote activation and BAX/BAK-induced cytochrome c release within a cell-free mitochondrial permeabilization assay [122]. This is also consistent with a role for direct mitochondrial/ER contacts in the generation and transfer of sphingolipids during cell death [122] (**Figure 3**). These data suggest that Drp1-stabilized apoptotic contact sites may be a conduit for lipid transfer from ER to mitochondria that may drive distinct processes in the release of cytochrome c.

## Conclusions.

We have summarized the emerging concepts in the cell biology that drives cell death, yet many questions remain. We propose a model whereby the activation of BAX/BAK leads to the stable assembly of Drp1- and MAPL-dependent ER/mitochondrial contacts that drive mitochondrial calcium entry (**Figure 1B-D**). The influx of calcium is a prerequisite for OPA1 oligomer disassembly and cristae remodeling, allowing the direct access of cytochrome c to the BAX/BAK channels (**Figure 1D, E**). There is also evidence to suggest that ER-mitochondrial contact sites may also facilitate sphingolipid exchange and accumulation of intermediates at mitochondria, which may play a role in the expansion of BAX/BAK channel, as an example (**Figure 3**).

To conclude, it has been established that mitochondria alter their shape during apoptosis, but it remained controversial whether these processes are functionally essential. We submit that the changes in mitochondrial architecture greatly facilitate the expansion of BAX/BAK channels and ultimate release of cytochrome c. There may be situations where

cells will die no matter what – a process referred to as “death by a thousand cuts”. It is the fine-tuning of these events that will define the tipping point to kill the cell or allow it to survive. Mechanistically, the discovery that ER contacts accompany the process of mitochondrial fission [11] led to new advances in understanding the apoptotic fission events as a highly stabilized contact, coordinated through the SUMOylation of Drp1. Understanding these mechanisms provides new molecular targets, particularly in the SUMOylation and deSUMOylation enzymes, that may open the door to new therapies in the treatment of cancer and degenerative diseases.

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## FIGURE LEGENDS

### Figure 1: Mechanism of mitochondria/ER contacts inducing cristae remodeling and cell death.

**A.** In healthy cells, the pro-apoptotic protein BAX and the pro-fission GTPase Drp1 are mainly cytosolic. **B.** After induction of apoptosis by different stimuli including staurosporin or expression of the cleaved-form of the BH3-only protein Bid, tBID, contacts between the ER and the mitochondria are increased. BAX is activated and recruited to the mitochondria. Both BAX and BAK are inserted into the OMM where they oligomerize. In parallel, Drp1 is dephosphorylated at serine 637 and phosphorylated at serine 616 leading to its specific recruitment at the mitochondria-ER contact points by its adaptors, MFF or MiDs, where it assembles into oligomers. In the death paradigm, Drp1 is then SUMOylated by MAPL, leading to its stabilization at the mitochondria/ER contacts. This results in prolonged mitochondrial constriction at apoptotic contact sites. The curvature induced through constriction has been shown to enhance the capacity of BAX/BAK to assemble into oligomers. **C.** The SUMOylation of Drp1 stabilizes a mitochondrial/ER platform facilitating ER- $Ca^{2+}$ transfer to the mitochondria (see text). Previous work has shown that cell death induces ER-$Ca^{2+}$ release through IP3R, which crosses the outer membrane via VDAC pores, and across the inner membrane through MCU. **D.** Finally, the apoptotic contact site co-ordinates the processes of mitochondrial constriction and calcium uptake, ultimately resulting in cristae remodeling and cytochrome c release. The metalloprotease zinc OMA1 cleaves the oligomeric, membrane-anchored form of OPA1, leading to the loss of cristae junctions, which drives the release of cytochrome c from the electron transport chain complexes into the cytosol. Recent data reveal an expanding, macromolecular BAX/BAK-channel within the OMM, seen as a ring-like structure allowing cytochrome c to cross the outer membrane. **E.** Mitochondrial fragmentation accompanies the release of the cytochrome c. Once in the cytosol the cytochrome c assembles into the apoptosome with the Apaf-1 and the caspase-9. This leads to the activation of the caspases-3/7 and their intracellular substrates degradation and finally cell death.

## Figure 2: Post-translational modifications of Drp1 in Apoptosis.

The activity of Drp1 and its recruitment to mitochondria to induce mitochondrial division is tightly regulated by post-translational modifications including S-nitrolysation, O-GlcNAcylation, ubiquitination, phosphorylation and SUMOylation [86]. These dynamic modifications couple mitochondrial architecture and dynamics to cell fate in a number of ways, from cell reprogramming [123•] to tumor growth [124,125], neuroprotection [126] and necrosis [127]. However, the phosphorylation at Serine 637 in human Drp1 plays a pivotal role in apoptosis. Indeed, Protein Kinase A (PKA) is recruited to mitochondria by the Protein Kinase A Anchor Protein 1 (AKAP1) leading to the Serine 637 phosphorylation of Drp1, suppression of its mitochondrial recruitment and GTPase activity, mitochondrial hyperfusion and inhibition of apoptosis [93,128]. In contrast, dephosphorylation of this serine by the phosphatase calcineurin induced Drp1 mitochondrial recruitment, mitochondrial fragmentation and sensitivity to cell death induction [92,93,129]. Interestingly, during apoptosis, AKAP1 is degraded and PKA recruitment at mitochondria is inhibited, thereby allowing the activation and recruitment of Drp1 [90]. However, these events may again be context specific, since hyperglycemia in a diabetic mouse model led to the activation of Rho-associated coiled coil-containing protein Kinase 1 (ROCK1), which phosphorylated Drp1 on the orthologous PKA serine residue (serine 600), yet in this context they observed mitochondrial fragmentation and apoptosis [130]. It is not enough to dephosphorylate the PKA site for Drp1 recruitment and activation, it is increasingly clear that a new phosphorylation of Drp1 at Serine 616 is important for mitochondrial fragmentation during cell death. During this process, this site is phosphorylated by the kinases Proteine Kinase C (PKC) [131] and the $Ca^{2+}$-/calmodulin-dependent kinase II (CaMKII) [132]. For example, during chronic β-adregenic receptor stimulation, CaMKII phosphorylates Drp1 at Serine 616 to induce mPTP opening and myocyte cell death [133•]. So far the data support the idea that dephosphorylation at Drp1 at serine 637 lie upstream of MAPL-dependent SUMOylation of Drp1, since knockdown of MAPL has no effect on AKAP1 degradation and loss of P-Drp1-S637 [7••,90].

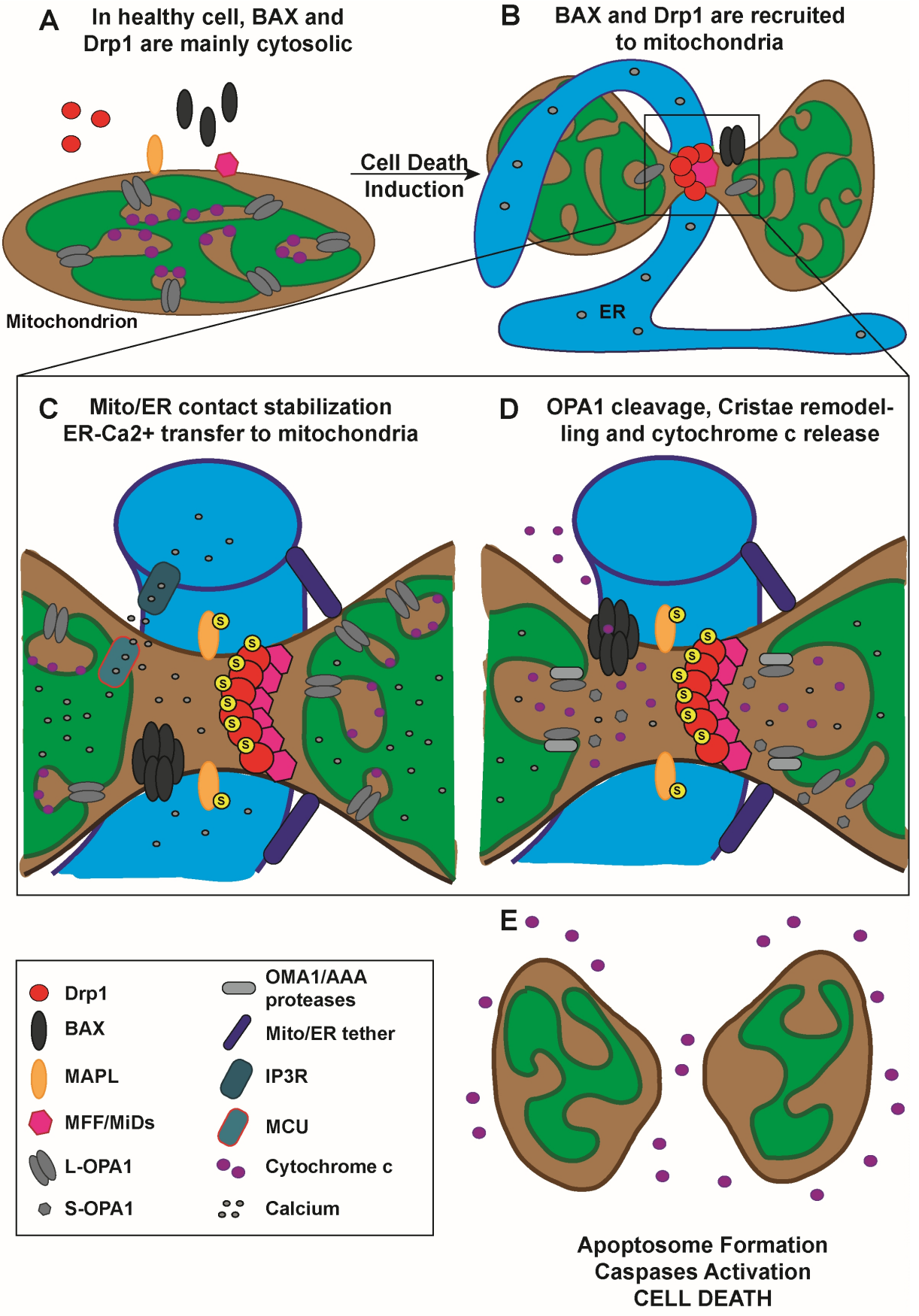
**Figure 3: Interconnection of the sphingolipid pathways and cell death at the mitochondria/ER contact sites.**

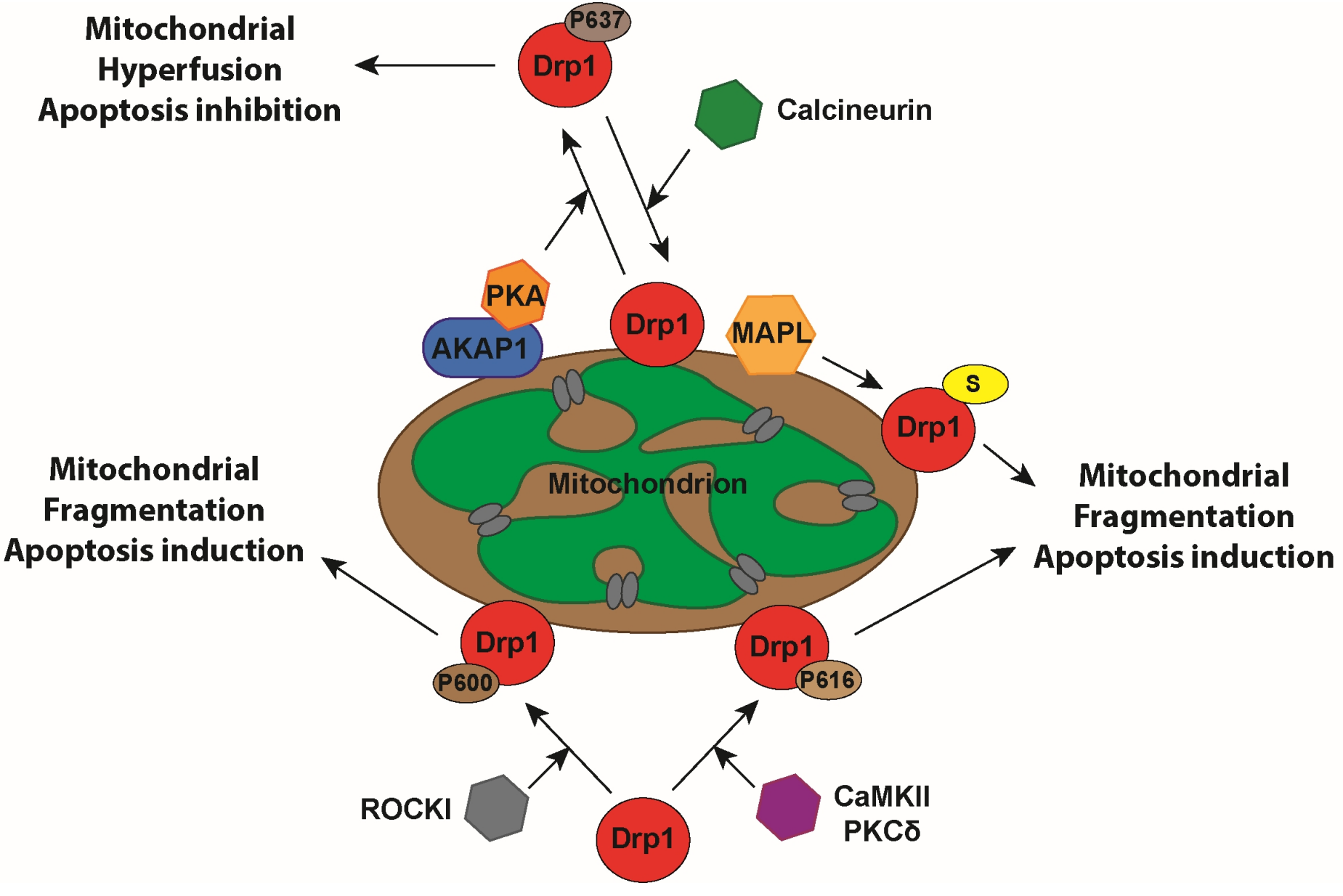
**A, B. The sphingomyelin pathway.** During cell death, the ER-localized sphingomyelin and its hydrolysis product, ceramide, are transferred to the mitochondria. Enzymes localized to mitochondria, including the sphingosine kinase 2 and the sphingosine-1-PO4, convert ceramide into sphingosine-1-phosphate and hexadecenal, respectively. These products were seen to promote the efficient OMM permeabilization by BAK **(A)** and BAX **(B)**, respectively, following BH3-only cell death induction.

**C, D. *De novo* pathway.** During cell death, ceramide synthase, which converts sphinganine into ceramide, has been shown to localized to mitochondria. The sphinganine is produced through a cascade of reactions initiating within the ER with the generation of 3-keto-sphinganine from Palmitoyl CoA and serine. We can hypothesis that sphinganine is transferred to mitochondria from the ER to produce ceramide via ceramide synthase activity. Interestingly, member of the Bcl-2 family can interfere in the generation of ceramide by direct interaction with the ceramide synthase. **(C)** Activated BAK was shown to be required for the activity of the ceramide synthase during cell death in an unknown mechanism. **(D)** Finally, the anti-apoptotic protein Bcl-2-L13, (also called Bcl-RAMBO) localized at the mitochondria-ER contact sites can directly bind ceramide synthase 2 and 6 at mitochondria to inhibit their enzyme activity delaying apoptosis (see text for details).

Cytochrome c release from cristae is ensured by the formation of proteins, lipids or proteins/lipids channels at the OMM. **(i)** Large lipid channels composed of hundreds of ceramide molecules, which pore diameter is sufficient to allow the release of cytochrome c. The activity of the channel is enhanced by the pro-apoptotic BAX and inhibited by the anti-apoptotic proteins, Bcl-2 and Bcl-xL. **(ii)** The most documented channel is composed of homo- or hetero-oligomers of the pro-apoptotic proteins, BAX and BAK. During apoptosis, activated BAX is recruited to mitochondria where it assembles, potentially with BAK, in a large ring-like structure in the OMM. The diameter of this BAX ring-structure is predominantly between 200 and 300 nm but larger diameter of 400 nm can be observed. Interestingly, the establishment of this BAX ring-like structure on fragmented mitochondria allowing the release of cytochrome c is Drp1-dependent. Finally, this structure may be assimilated as channel since the

enclosed area is devoid of OMM proteins. **(iii)** The third hypothesis is the formation of a mix channel composed of BAX/BAK proteins and sphingolipid-intermediates.


A
In healthy cell, BAX and Drp1 are mainly cytosolic
Mitochondrion
Cell Death Induction
B
BAX and Drp1 are recruited to mitochondria
ER
C
Mito/ER contact stabilization
ER-Ca2+ transfer to mitochondria
D
OPA1 cleavage, Cristae remodelling and cytochrome c release
S
Drp1
BAX
MAPL
MFF/MiDs
L-OPA1
S-OPA1
OMA1/AAA proteases
Mito/ER tether
IP3R
MCU
Cytochrome c
Calcium
E
Apoptosome Formation
Caspases Activation
CELL DEATH


Mitochondrial
Hyperfusion
Apoptosis inhibition
P637
Drp1
Calcineurin
PKA
AKAP1
Drp1
MAPL
S
Drp1
Mitochondrial
Fragmentation
Apoptosis induction
Mitochondrion
Mitochondrial
Fragmentation
Apoptosis induction
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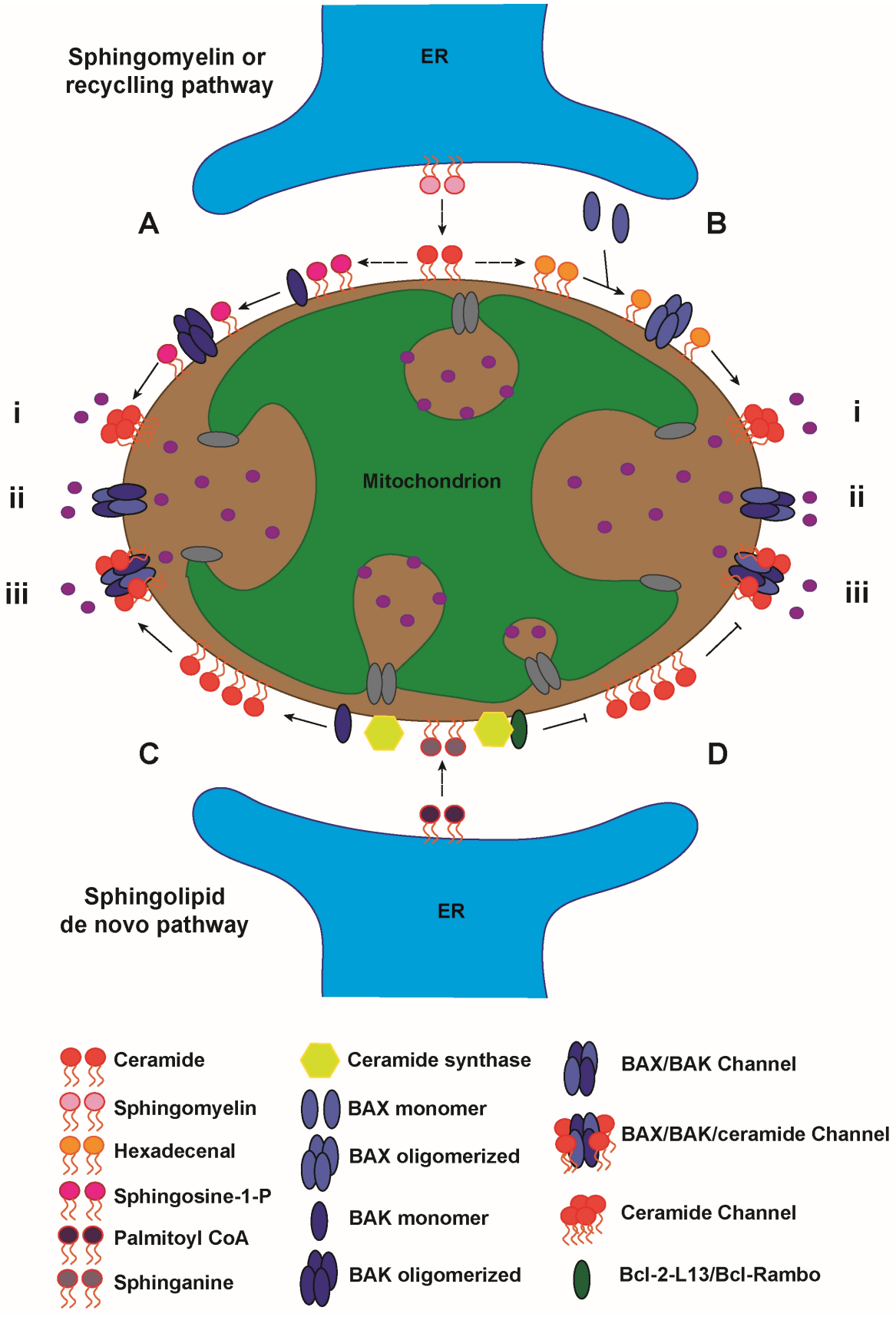
P600
Drp1
P616
ROCKI
Drp1
CaMKII
PKCδ


Sphingomyelin or
recyclling pathway
ER
A
B
i
ii
iii
Mitochondrion
i
ii
iii
C
D
Sphingolipid
de novo pathway
ER
Ceramide
Sphingomyelin
Hexadecenal
Sphingosine-1-P
Palmitoyl CoA
Sphinganine
Ceramide synthase
BAX monomer
BAX oligomerized
BAK monomer
BAK oligomerized
BAX/BAK Channel
BAX/BAK/ceramide Channel
Ceramide Channel
Bcl-2-L13/Bcl-Rambo

## HIGHLIGHTS

- Drp1-SUMOylation stabilizes the mitochondrial/ER interface required for apoptosis
- This mitochondrial/ER platform facilitates calcium and lipid fluxes during cell death
- Drp1 and mitochondrial fragmentation are crucial for cristae remodelling during apoptosis
- OPA1-oligomer cleavage and cristae remodelling are Ca2+-dependent