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# Mitochondrial Dynamics and Apoptosis: A Painful Separation

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**Reporting in *Molecular Cell*, Sheridan et al. (2008) and Breckenridge et al. (2008) show that mitochondrial fragmentation is not required to induce cell death. Meanwhile, Yamaguchi et al. show that proapoptotic Bcl-2 family members promote cytochrome *c* mobilization through Opa1-mediated cristae remodeling. Therefore, the connection between mitochondrial structure and apoptosis is more complex than previously imagined.**

Mitochondria are known to form a filamentous network that extends throughout the cell. The dynamics of this network are regulated by the proteins Drp1 and Fis1, which control the fission of the outer mitochondrial membrane, and Opa1, which is required for the fusion of the inner membrane. Following an apoptotic signal, Drp1 translocates from the cytosol to the mitochondria and triggers mitochondrial fission prior to caspase activation. At the mitochondrial fission sites, Drp1 colocalizes with the proapoptotic Bcl-2 family members Bax and Bak, which are responsible for mitochondrial outer membrane permeabilization (MOMP). Inhibiting the fission process by downregulating Fis1 or Drp1 activity has been purported to alter cytochrome *c* release and delay apoptosis, suggesting that Drp1-induced mitochondrial fission plays a role in MOMP and the release of cytochrome *c* (Suen et al., 2008). Now Sheridan et al. (2008) report that, in mammalian cells, mitochondrial fission is not required for MOMP and apoptosis.

Overexpression of fission proteins is known to cause cell death that can be inhibited by antiapoptotic Bcl-2 family members without affecting the transition from the tubular to punctate mitochondrial morphology. This suggested that fragmentation and apoptosis could be uncoupled (Arnoult, 2006). Sheridan et al. (2008) have comprehensively investigated this issue using fragmentation induced by Bcl-$x_L$ or Mcl-1—antiapoptotic BH3-domain-containing proteins of the Bcl-2 family. Using fluorescent recovery after photobleaching to monitor mitochondrial fragmentation, they showed that mitochondria were disconnected following Bak or Bax overexpression. Although Bcl-$x_L$ or Mcl-1 could inhibit the release of apoptotic markers cytochrome *c* and Smac/DIABLO within these Bax/Bak-overexpressing cells, the fragmentation persisted, thus indicating that MOMP and mitochondrial fission were separable events. Interestingly, they also showed that Bcl-$x_L$ on its own appeared to disrupt mitochondrial morphology and that both proapoptotic and antiapoptotic Bcl-2 family members—in addition to affecting mitochondrial fragmentation—may have different targets within the machinery that governs the movement of mitochondria in cells.

Experiments performed by Breckenridge et al. (2008) in *C. elegans* led to a similar conclusion. The fission and fusion machinery is broadly conserved in *C. elegans* and even though MOMP has not been observed, mitochondrial fragmentation occurs in cells expressing the BH3-only proapoptotic protein EGL-1, which are destined to undergo developmental cell death (Jagasia et al., 2005). However, the role of the fusion and fission genes in apoptosis remained to be characterized. Breckenridge et al. showed that mutants in mitochondrial fusion (*fzo-1*, *eat-3*) and fission (*Drp1*) genes were embryonic lethal, yet mutants in the known orthologs of hFis1 (*fis1* and *fis2*) were viable. Studying the mitochondria in the early embryo showed that the fusion mutants gave rise to a fragmented mitochondrial network, whereas the *Drp1* mutant displayed highly fused mitochondria. Yet developmental cell death in these mutants was found to occur normally. This contrasts with a recent paper (Jagasia et al., 2005) that showed that an overexpressed Drp1 mutant (Drp1-K40A) increased the number of surviving cells during development. However, in a mutant

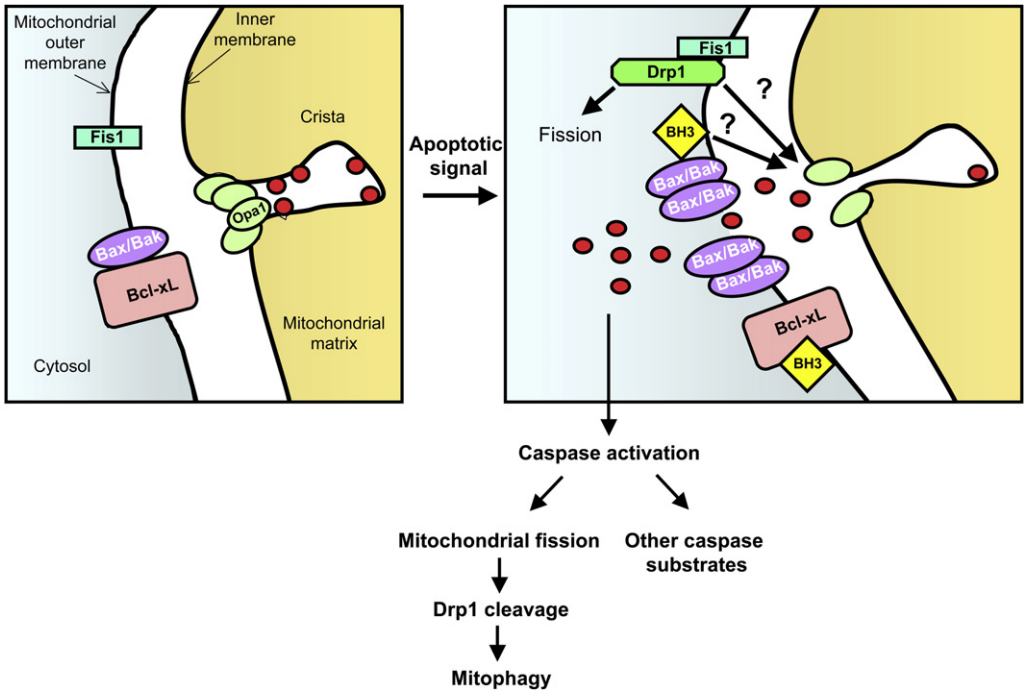


**Figure 1. Mitochondrial Fission and Fusion Proteins in Mammalian Apoptosis**
In healthy mitochondria, cytochrome *c* (red circles) is mostly confined to the cristae, and a complex of Opa1 (green ovals) appears to maintain a wide "neck" at the cristae. Proapoptotic members of the Bcl-2 family (Bax/Bak) are kept inactive. In the presence of an activating BH3 domain and Bax or Bak, signals are relayed, possibly by fission proteins in the outer membrane, to the Opa1 complex, which disassembles and allows cytochrome *c* to exit the cristae. Bax or Bak oligomerize in the mitochondrial outer membrane to permeabilize it and allow the proteins in the inter membrane space to escape. Permeabilization can be inhibited by antiapoptotic Bcl-2 family members. Based on findings in *C. elegans*, subsequent caspase activation could lead to cleavage of Drp1, which may promote clearance of the mitochondria, possibly by mitophagy.

*ced-3* background, where caspase activation is reduced and any survival effect enhanced, loss of *Drp1* or *fis2* increased the number of extra cells in the anterior pharynx. Moreover, double mutants of *fis2* and *Drp1* showed an additive effect indicating that although they can interact in higher organisms to promote mitochondrial fission, these two genes can act independently during cell death in *C. elegans*. Surprisingly, both genes were found to function downstream of *ced-3*, and Drp1 at least was found to be a substrate of the CED-3 caspase. The authors also present evidence suggesting that the proapoptotic role of Drp1 is distinct from its role in mitochondrial fission. Interestingly, whereas mitochondria were found to be eliminated in apoptotic cells (a process known as mitophagy), they persisted in cells lacking functional Fis2 or Drp1. Since mitochondrial fission occurs normally in *fis2*-deficient worms, it is unlikely that the fission of mitochondria alone is responsible for their elimination and it is therefore unclear how Fis2 and cleaved Drp1 stimulate mitophagy. Elimination of cytochrome *c*-depleted mitochondria has been previously reported in mammalian cells when caspases are inactivated (Xue et al., 2001), and alternative roles for mitochondrial fission in apoptosis have been proposed (Parone and Martinou, 2006). It will be interesting to test whether, under these particular conditions in mammalian cells, Drp1 or Fis1 is responsible for mitophagy. Endophilin B1, a protein that interacts with Bax and plays a role in autophagy, could therefore be a connector between Drp1, Bax, and the autophagy machinery (Suen et al., 2008).

Cytochrome *c* release in itself is a contentious issue. It has been widely held that two distinct pools of cytochrome *c* exist, with the larger pool held within the mitochondrial cristae, which are folds of the mitochondrial inner membrane. The release of the large pool of cytochrome *c* is thought to require a change in the structure of the cristae. Scorrano and colleagues have previously investigated the role of Opa1 oligomerization in the control of cristae remodeling. They have also shown that the proapoptotic BH3-only protein Bid (tBid) causes alterations in the cristae structure, widening them to facilitate cytochrome *c* release, although this appeared to be independent of its BH3 domain (Scorrano et al., 2002). Yamaguchi et al. here provide evidence that the BH3 domain of Bid or Bim, together with Bax and Bak, is required for the cristae junction remodeling that would mobilize cytochrome *c* for release through the outer mitochondrial membrane. Surprisingly, rather than undergoing a major reorganization and wide opening, the cristae junctions became narrower as a result of Opa1 disassembly. This concurs with a recent study that suggested that any major conformational changes in cristae structure may occur as later, caspase-dependent events (Sun et al., 2007). To study the requirement of Opa1 assembly in cristae conformation, the authors mutated the GTPase domain of Opa1 to create a mutant that possessed enhanced binding to itself. If Opa1 disassembly were inhibited, then cytochrome *c* would be expected to remain within the cristae and apoptosis inhibited. Opa1 Q297V blocked BimS-induced apoptosis downstream of Bax/Bak activation, and importantly, Omi/HtrA2 and Smac/DIABLO

were retained, along with cytochrome *c* within the mitochondria despite MOMP. The Opa1 mutant provides an interesting tool to further explore the mysteries that link Bcl-2 family members, Opa1, and cristae remodeling.

These three papers help to tease out the intricacies between the mitochondrial fission and fusion machinery and apoptosis. Although apoptotic mitochondrial fission appears to be unnecessary for caspase activation (Yamaguchi et al., 2008; Sheridan et al., 2008), components of the fission and fusion machinery were found to play a role in the release of mitochondrial activators of caspases in mammalian cells (Yamaguchi et al., 2008) and in mitophagy in *C. elegans* (Breckenridge et al., 2008). These findings suggest that these proteins, possibly through post-translational modifications such as cleavage of Drp1 in *C. elegans*, could endorse novel functions during apoptosis (see Figure 1). In support of this hypothesis, it was recently reported that a Drp1 chemical inhibitor can prevent MOMP in isolated mitochondria when mitochondrial fission is unlikely to occur (discussed in Suen et al. [2008]). It remains to be understood how the components of the fission and fusion machinery are functioning in coordination with members of the Bcl-2 family. Given the role of the mitochondrial dynamins (Drp1 and Opa1) on the shaping of mitochondrial membranes, it is likely that particular membrane structures mediate some interaction between Bcl-2 family members and proteins of the fission and fusion machinery.

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