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New role of the BCL2 family of proteins in the regulation of mitochondrial dynamics

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Abstract

Mitochondria are highly dynamic organelles that constantly fuse and divide. Dynamin-related GTPases are the core components of the machineries that mediate mitochondrial fusion and fission. The role and regulation of these machineries are currently under intense investigation. Recently, members of the BCL2 family of proteins, conserved regulators of apoptosis, have been implicated in the regulation of mitochondrial dynamics. Here, we review the functions of mitochondrial fusion and fission in apoptotic and non-apoptotic cells and how members of the BCL2 family of proteins regulate these functions.

Introduction

Just when we thought that we knew everything about the ‘power plant’ of the cell, mitochondria were shown to play crucial roles in several processes other than that of energy production, such as signaling and apoptosis [1,2]. In addition, recent advances in live cell imaging revealed that mitochondria are not small ‘bean shaped’ but highly dynamic organelles that constantly fuse and divide and can form extensive networks. Dynamin-related GTPases are the central components of the mitochondrial fusion and fission machineries and are conserved from yeast to humans. Specifically, two dynamin-related GTPases are required for mitochondrial fusion [3]. Mammalian Mitofusin 1 and 2 (Mfn1, 2), *C. elegans* FZO-1 and *D. melanogaster* Fzo and dMfn are required for the fusion of the outer-mitochondrial membrane (OMM). Mammalian Opa1, *C. elegans* EAT-3 and *D. melanogaster* Opa1 are required for the fusion of the inner-mitochondrial membrane (IMM). Mitochondrial fission requires only one dynamin-related GTPase, namely Drp1 in mammals, DRP-1 in worms and Drp1 in flies [4]. Below, we discuss the importance of these proteins in apoptotic and non-apoptotic cells. We also discuss the newly discovered functions of members of the BCL2 family of proteins (Box 1), important regulators of apoptosis, in the regulation of mitochondrial dynamics.

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Mitochondrial dynamics and apoptosis

Mitochondrial fission in apoptosis

In mammalian cells, mitochondria play a central role in apoptosis by releasing several pro-apoptotic factors located in the intermembrane space, such as cytochrome c [2]. In 2000, Desagher et al first reported that mitochondria undergo excessive fragmentation during apoptosis [5]. A year later, Frank et al discovered that reducing mitochondrial fission by expressing a dominant-negative mutant of Drp1 was able to block apoptosis-induced mitochondrial fragmentation, cytochrome c release and apoptosis [6], assigning for the first time a role for Drp1-like proteins in apoptosis. This finding has been supported by several independent studies [7,8] but challenged by others [9,10]. The current consensus is that blocking Drp1 activity does not block but delay apoptosis. Drp1 is therefore important for efficient execution of apoptosis in mammalian cells.

Jagasia et al showed that DRP-1 is important for efficient execution of apoptosis in *C. elegans* as well [11]. Whereas over-expressing wild-type DRP-1 causes mitochondrial fragmentation and ectopic cell death in the absence of apoptotic signals, over-expressing a dominant negative mutant of DRP-1 blocks mitochondrial fragmentation and prevents the death of about 20% of the cells [11]. Recently, a weak loss-of-function mutation in the caspase gene *ced-3*, which causes a partial block of cell death, has been shown to be enhanced by a *drp-1* loss-of-function mutation, confirming that DRP-1 has pro-apoptotic function [12; Lu, Y and Conradt, B., unpublished observation]. Drp1-mediated mitochondrial fragmentation has also been observed during the apoptotic process in *D. melanogaster* [13]. Studies with primary cell cultures have revealed that depletion of Drp1 confers resistance to a variety of apoptotic stimuli [13]. Consistently, *drp1* mutant embryos are less sensitive to irradiation-induced apoptosis [14]. Hence, Drp1 has pro-apoptotic activity also in flies.

Mitochondrial fusion in apoptosis

Even though it is uncertain whether the over-expression of Mfn1 or Mfn2 protects mammalian cells against apoptosis, it is clear that depletion of Mfns does not cause mammalian cells to die in the absence of apoptotic stimuli [15,16]. On the contrary, depletion of Opa1 causes 25–35% of cells to die spontaneously [17]. However, Opa1's role in apoptosis does not seem to be related to its function in mitochondrial fusion but to its role in the maintenance of *cristae*, invaginations of the IMM [18,19].

Whereas it remains unknown whether the mitochondrial fusion machinery participates in apoptosis in *D. melanogaster*, in *C. elegans* it has been shown that neither *fzo-1* nor *eat-3* plays a role in apoptosis [12,20, Rolland, S. and Conradt, B., unpublished observation].

Is the role of Drp1-like proteins in apoptosis dependent on their role in mitochondrial fission?

Whether the pro-apoptotic activity of Drp1-like proteins is related to their role in mitochondrial fission is still under debate. If mitochondrial fragmentation *per se* is required for apoptosis induction, one would expect that blocking mitochondrial fusion would also cause apoptosis. However, blocking fusion at most sensitizes cells to apoptotic stimuli. In addition, a recent study using a small molecule inhibitor of Drp1 showed that the role of Drp1 in fission and apoptosis can be separated [21]. Altogether, these observations point toward a role of Drp1-like proteins in apoptosis independent of their role in mitochondrial fission.

BCL2 proteins control mitochondrial dynamics in apoptotic cells

In mammals, over-expression of the pro-apoptotic BCL2 family member Bax promotes Drp1-dependent mitochondrial fragmentation [6]. Interestingly, during apoptosis, Bax co-localizes with Drp1 and Mfn2 at sites on mitochondria where fission subsequently occurs [22]. In addition, Bax translocation to mitochondria correlates with a reduction in mitochondrial fusion [23]. Hence, BCL2 proteins cause mitochondrial fragmentation by activating fission and/or blocking fusion. This model is supported by two recent findings. In apoptotic cells, Bax and Bak promote mitochondrial fragmentation by stabilizing the association of Drp1 with mitochondria [24]. Furthermore, Bak regulates mitochondrial dynamics by interacting with Mfns [25]. The pro-apoptotic BH3-only proteins have also been implicated in the regulation of mitochondrial dynamics during apoptosis. For example, Noxa and Puma have been shown to trigger Drp1-dependent mitochondrial fragmentation [16,26]. In *C. elegans*, the pro-apoptotic complex of the BCL2-like protein CED-9 and the BH3-only protein EGL-1 is necessary for DRP-1-dependent mitochondrial fragmentation during apoptosis [11]. In *D. melanogaster*, the BCL2 homologs DeBCL and Buffy do not seem to control mitochondrial dynamics in dying cells. However, the pro-apoptotic regulators Reaper and Hid have been shown to trigger Drp1-dependent fragmentation during apoptosis [14]. In summary, pro-apoptotic members of the BCL2 family of proteins are involved in causing mitochondrial fragmentation in apoptotic cells (Figure 1, Table 1).

Mitochondrial dynamics in non-apoptotic cells

Mitochondrial fission in non-apoptotic cells

drp1 mutations cause embryonic lethality in *C. elegans* and *D. melanogaster* [27,28]. In mouse, Drp1 is essential for brain development [29,30]. Recently, it was shown that a patient with a broad range of abnormalities such as defects in neuronal development is heterozygous for a dominant-negative allele of Drp1 [31]. Interestingly, primary neurons of Drp1 knockout mice form fewer neurites and synapses than control cells [29]. Consistently, Drp1-mediated mitochondrial fission plays an important role in the localization of mitochondria to pre-synaptic buttons and post-synaptic dendritic spines, both of which are required for synapse formation [32,33]. Similarly, the loss of *drp1* function in *D. melanogaster* impairs synaptic localization of mitochondria to neuromuscular junctions, which results in several neurological defects [28]. The mitochondrial fission machinery therefore plays an important role during the development of the nervous system.

Studies in *C. elegans* and mouse embryonic fibroblasts also revealed that Drp1-mediated mitochondrial fission is necessary to achieve equal segregation of mitochondria between two daughter cells during cell division [27,29]. In HeLa cells it has been observed that mitochondria form a filamentous network during interphase, fragment early during mitosis and reform a tubular network after cytokinesis [34]. These cell cycle-dependent changes in mitochondrial morphology seem to be mediated by phosphorylation of Drp1 by Cdk1/cyclin B [34].

Mitochondrial fusion in non-apoptotic cells

The fusion machinery plays an important role in embryonic development in *C. elegans*, *D. melanogaster* and mammals [12,20,35,36]. In mammals, mitochondrial fusion has been shown to play a particularly important role in neurons. Specifically, the neurodegenerative diseases Charcot-Marie-Tooth disease 2a (CMT2a) and Autosomal Dominant Optic Atrophy (ADOA) can be caused by mutations in Mfn2 and Opa1, respectively [37]. Why these mutations only affect neurons is currently being elucidated. CMT2a affects primarily peripheral neurons, in which mitochondria have to travel long distances to reach synapses.

Interestingly, fusion-deficient mitochondria have been shown to have decreased mobility [35] and Mfn2 has recently been implicated in axonal transport of mitochondria [38].

Mitochondrial fusion also participates in the maintenance of the mitochondrial genome (mtDNA). Unlike bacterial division, which is coupled with DNA replication, mitochondrial fission does not ensure that both daughter organelles inherit mtDNA [39]. Mitochondrial fusion has therefore been proposed to allow the rescue of mtDNA-deficient mitochondria [39]. Conditional Mfn1, 2 knockout in skeletal muscle cells support this model and demonstrate that mitochondrial fusion is also important to prevent the accumulation of mutated mtDNA with increasing age [40].

Finally, mitochondrial fusion also plays a role during cell division. In mammalian cells, mitochondria undergo hyperfusion during the G1-S transition. This hyperfusion seems to control cell cycle progression by regulating the level of cyclin E [41].

BCL2 proteins modulate mitochondrial dynamics in non-apoptotic cells

Pro-apoptotic Bax and Bak were the first BCL2 proteins discovered to play a role in mitochondrial dynamics in non-apoptotic cells. Indeed, Bax/Bak knockout cells have mitochondrial fusion defects [42]. In *C. elegans*, the anti-apoptotic BCL2-like protein CED-9 has recently been shown to promote mitochondrial fusion in embryonic and muscle cells [43,44]. Expression of CED-9 in mammalian cells causes mitochondrial fusion as well, indicating that the pro-fusion activity of BCL2 proteins is conserved [45]. The function and regulation of these fusion events remain to be determined. In mammalian neurons, anti-apoptotic BCL-xl has been shown to play a role in synaptogenesis by promoting Drp1-mediated mitochondrial fission [33]. In addition, BCL-xl has recently been shown to also affect the rate of mitochondrial fusion [46]. Hence, whereas BCL2 proteins only have pro-fission activity in apoptotic cells, they appear to have both pro-fission and pro-fusion activities in non-apoptotic cells (Table 1). What determines which of these two processes they promote remains to be elucidated.

Mechanisms of regulation of the mitochondrial fusion and fission machineries by BCL2 proteins

BCL2 proteins regulate the mitochondrial fusion machinery by directly interacting with its components. In mammalian cells, Bax, Bak, BCL2 and BCL-xl have been shown to interact with Mfns [25,42,45]. In *C. elegans*, CED-9 interacts with FZO-1. This interaction is required for CED-9's pro-fusion activity and involves a domain of FZO-1 predicted to form a coiled-coil structure [44]. Interestingly, the corresponding domain of Mfn2 is also required for the interaction with Bax [42], suggesting that the function of this domain is evolutionary conserved. How these interactions affect the function of mitochondrial fusion proteins is starting to be elucidated. Bax and Bak have been proposed to promote mitochondrial fusion by regulating the sub-mitochondrial localization of Mfn2, its mobility in the OMM as well as its ability to oligomerize [42].

BCL2 proteins also interact with mitochondrial fission proteins. For example, BCL-xl interacts with Drp1 in neurons and is thought to increase the GTPase activity of Drp1 *in vitro* [33]. Even though it is still unknown whether Bax or Bak interact with Drp1, it has recently been shown that Drp1 is sumoylated during apoptosis in a Bax/Bak-dependent manner and that this modification causes the stable association of Drp1 with mitochondrial membranes [24]. Similarly, Puma has been shown to enhance the recruitment of Drp1 to mitochondria during apoptosis, however the mechanism through which it does so is unknown [47].

BCL2 proteins can therefore control mitochondrial fusion and fission proteins by affecting their localization, mobility, oligomerization and/or GTPase activities. However, more studies are needed to fully understand the contribution of each of these mechanisms to the ability of BCL2 proteins to control mitochondrial dynamics.

Summary and future perspectives

Mitochondrial fusion and fission play important roles in apoptotic and non-apoptotic cells. As described above, some of these roles are under the control of the BCL2 family of proteins (Figure 2). Members of this family have been associated with several cancers and have therefore become a prime target for cancer therapeutics. However, these therapeutics are designed assuming that BCL2 proteins specifically function in apoptosis. In light of the new roles of BCL2 proteins in mitochondrial dynamics, they should therefore be re-evaluated. More studies are clearly needed to fully understand the functions of BCL2 proteins in mitochondrial dynamics. While it is clear that apoptotic signals are the signals sensed by BCL2 proteins in dying cells, the signals sensed by BCL2 proteins in non-apoptotic cells remain to be identified. In addition, BCL2 proteins have been shown to be able to promote both mitochondrial fusion and fission. Hence, another future challenge is to understand how those two opposite activities are regulated.

Box 1: BCL2 proteins are crucial and conserved regulators of apoptosis

The BCL2 family of proteins is composed of anti- and pro-apoptotic proteins. Anti-apoptotic BCL2 proteins (such as BCL2 or Bcl-xl in mammals and CED-9 in *C. elegans*) share four BCL2 homology or 'BH' domains (BH1–BH4). Pro-apoptotic BCL2 proteins can be separated into two subclasses: the Bax-like proteins (such as Bax and Bak in mammals – no Bax-like proteins have been identified in *C. elegans*), which contain three BH domains (BH1–BH3) and the BH3-only proteins (such as Puma or Noxa in mammals and EGL-1 in *C. elegans*), which contain a single BH domain (BH3). In mammals, the BCL2 family of proteins controls the integrity of the outer-mitochondrial membrane (OMM) (see for review: [48]). During apoptosis, permeabilization of the OMM leads to release of cytochrome c. Released cytochrome c binds to Apaf-1 triggering the formation of the apoptosome and the processing of caspase-9, thereby leading to apoptosis. In *C. elegans*, the anti-apoptotic BCL2 protein CED-9 sequesters the Apaf-1-like protein CED-4 to mitochondrial membranes in non-apoptotic cells. In apoptotic cells, binding of the BH3-only protein EGL-1 to CED-9 disrupts CED-9's interaction with CED-4. Released CED-4 oligomerizes and activates the processing of the caspase CED-3 and CED-3-dependent apoptosis. When bound to EGL-1, CED-9 has also been proposed to adopt a pro-apoptotic Bax-like conformation (see for review: [49])

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Figure 1. Mitochondrial dynamics and apoptosis in worms, flies and mammals

In *C. elegans* the pro-apoptotic complex of CED-9 and EGL-1 promotes Drp-1-dependent mitochondrial fragmentation in apoptotic cells. During *D. melanogaster* apoptosis, Drp-1-mediated mitochondrial fragmentation is under the control of the pro-apoptotic proteins Reaper (Rpr) and Hid but not the BCL2 homologs DeBCL and Buffy. In mammals, multiple sets of proteins have been shown to promote Drp-1-mediated mitochondrial fragmentation in dying cells. The pro-apoptotic BCL2 family members Bax/Bak stabilize Drp1 on the mitochondrial surface and the Bak/Mfn complex normally present in non-apoptotic cells is disrupted during apoptosis. In addition, the BH3-only proteins Puma and Noxa activate Drp1 by a mechanism that remains to be determined. In all three model systems, it is still under debate whether the pro-apoptotic functions of Drp1-like proteins are related to their roles in mitochondrial dynamics.

Figure 2. The BCL2 family of proteins regulates mitochondrial dynamics in apoptotic and non-apoptotic cells

In apoptotic cells, pro-apoptotic BCL2-like proteins promote mitochondrial fragmentation by activating Drp1-like proteins and potentially blocking Mfn-like and Opa1-like proteins. Some evidences indicate that Drp1-like and Opa1-like proteins promote apoptosis independently of their role in mitochondrial dynamics. In non-apoptotic cells, both pro- and anti-apoptotic BCL2 proteins promote mitochondrial fusion or fission depending on the cellular context.

Table 1

Summary of the pro-fusion and pro-fission activities of the BCL2 family of proteins in apoptotic (red) and non-apoptotic (green) cells.

Organism	Activity	Protein	Role
<i>C. elegans</i>	Pro-fission	CED-9/EGL-1[11]	Promotes mitochondrial fragmentation in apoptotic cells
	Pro-fusion	CED-9[43,44]	unknown
Mammals	Pro-fission	Bax/Bak[6,16,22–25]	Promotes mitochondrial fragmentation in apoptotic cells
		Puma[16,47]	Promotes mitochondrial fragmentation in apoptotic cells
		Noxa[26]	Promotes mitochondrial fragmentation in apoptotic cells
		Bcl-xl[33,46]	Promotes Drp1-dependent synaptogenesis
	Pro-fusion	Bax/Bak[42]	unknown
		BCL2[16,45]	unknown
		Bcl-xl[16,45,46]	unknown