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The mitochondria–endoplasmic reticulum contact sites: a signalling platform for cell death

Julien Prudent[1] and Heidi M McBride[2]

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.

Addresses
[1] Medical Research Council, Mitochondrial Biology Unit, University of Cambridge, Wellcome Trust/MRC building, Cambridge Biomedical Campus, Hills Road, Cambridge, CB2 0XY, UK
[2] Montreal Neurological Institute, McGill University, 3801 University Avenue, Montreal, QC H3A 2B4, Canada

Corresponding authors: Prudent, Julien (julien.prudent@mrc-mbu.cam.ac.uk), McBride, Heidi M (heidi.mcbride@mcgill.ca)

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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/mitochondria contacts, including the machinery that regulates mitochondrial fission and fusion [5]. First, the fusion GTPase Mitofusin 2 (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 the pro- and the anti-apoptotic proteins of this family controls 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 complexes 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 mitochondrial contact site and cristae-organizing system (MICOS) complex and the profusion GTPase Optic Atrophy 1 (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 [35–37,38•]. 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 but before 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 Small Ubiquitin-related MOdifier (SUMO)-ylation 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 [11,12,13••,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 ~40 kDa 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].