Mitochondria and cell death: outer membrane permeabilization and beyond

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Abstract | Mitochondrial outer membrane permeabilization (MOMP) is often required for activation of the caspase proteases that cause apoptotic cell death. Various intermembrane space (IMS) proteins, such as cytochrome *c*, promote caspase activation following their mitochondrial release. As a consequence, mitochondrial outer membrane integrity is highly controlled, primarily through interactions between pro- and anti-apoptotic members of the B cell lymphoma 2 (BCL-2) protein family. Following MOMP by pro-apoptotic BCL-2-associated X protein (BAX) or BCL-2 antagonist or killer (BAK), additional regulatory mechanisms govern the mitochondrial release of IMS proteins and caspase activity. MOMP typically leads to cell death irrespective of caspase activity by causing a progressive decline in mitochondrial function, although cells can survive this under certain circumstances, which may have pathophysiological consequences.

Apoptosis is a genetically encoded programme leading to cell death that is involved in normal development and homeostasis throughout the animal kingdom. Deregulated apoptosis has been implicated in diverse pathologies, including cancer and neurodegenerative disease. The defining morphological characteristics of apoptosis include cell shrinkage, nuclear fragmentation, chromatin condensation and membrane blebbing, all of which are due to the proteolytic activity of the caspase proteases[1,2] (BOX 1; see Supplementary information S1 (movie)). Caspases orchestrate apoptosis through the cleavage of numerous proteins, ultimately leading to the phagocytic recognition and engulfment of the dying cell.

In vertebrate cells, apoptosis typically proceeds through one of two signalling cascades termed the intrinsic and extrinsic pathways, both of which converge on activating the executioner caspases, caspase 3 and caspase 7 (FIG. 1). In the intrinsic pathway, mitochondrial outer membrane permeabilization (MOMP), which leads to the release of pro-apoptotic proteins from the mitochondrial intermembrane space (IMS), is the crucial event driving initiator caspase activation and apoptosis (BOX 2). Following its release from mitochondria, cytochrome *c* binds apoptotic protease-activating factor 1 (APAF1), inducing its conformational change and oligomerization and leading to the formation of a caspase activation platform termed the apoptosome. The apoptosome recruits, dimerizes and activates an initiator caspase, caspase 9, which, in turn, cleaves and activates caspase 3 and caspase 7. Mitochondrial release of second mitochondria-derived activator of caspase (SMAC; also known as DIABLO) and OMI (also known as HTRA2) blocks X-linked inhibitor of apoptosis protein (XIAP)-mediated inhibition of caspase activity. MOMP is a highly regulated process, primarily controlled through interactions between pro- and anti-apoptotic members of the B cell lymphoma 2 (BCL-2) family (BOX 3).

In the extrinsic pathway, death receptor ligation causes the recruitment of adaptor molecules, such as FAS-associated death domain protein (FADD), that bind, dimerize and activate an initiator caspase, caspase 8. Active caspase 8 directly cleaves and activates the executioner caspases, caspase 3 and caspase 7. In so-called type I cells, caspase 8-mediated activation of the executioner caspases is sufficient to induce apoptosis in the absence of MOMP. Crosstalk between the extrinsic and intrinsic pathways occurs through caspase 8-mediated cleavage of BCL-2 homology 3 (BH3)-interacting domain death agonist (BID; a BH3 domain-only protein), leading to BID activation and MOMP. This step is crucial for death receptor-induced apoptosis in type II cells. The requirement for MOMP-induced XIAP antagonism discriminates between type I and type II cells in death receptor-mediated apoptosis. Hepatocytes are an *in vivo* type II cell; injection of wild-type mice with the FAS death receptor ligand, FASL,

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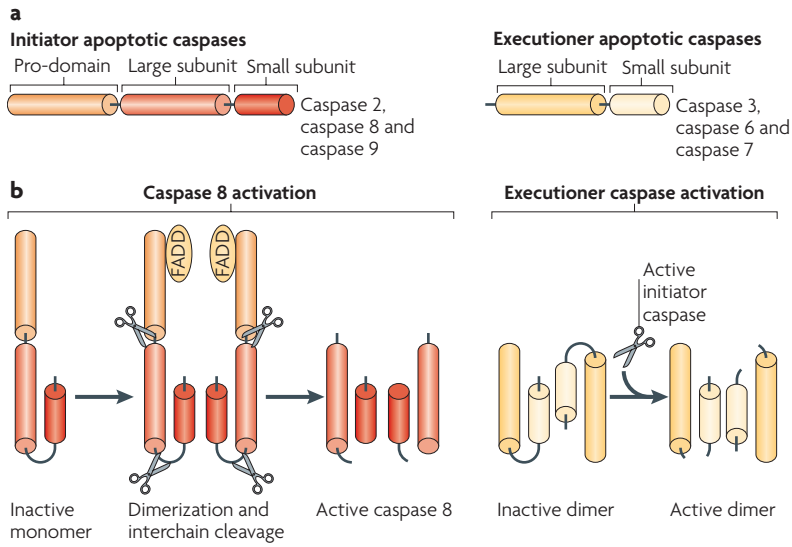
Box 1 | **Caspase classification and activation**



Caspases (Cys Asp acid proteases) cleave substrates in a highly specific manner after the Asp residue in short tetrapeptide (X-X-X-Asp) motifs. Besides apoptotic roles, some caspase family members have non-apoptotic functions in processes such as cytokine maturation, inflammation and differentiation. Additionally, apoptotic caspases can have non-apoptotic roles in certain circumstances[128–130]. Apoptotic caspases can be divided into two classes: initiator and executioner caspases (see the figure, part **a**). Initiator caspases (caspase 2, caspase 8 and caspase 9) are the apical caspases in apoptosis signalling cascades and their activation is normally required for executioner caspase (caspase 3, caspase 6 and caspase 7) activation. The repertoire of initiator caspase substrates is limited and includes self-cleavage, BCL-2 homology 3 (BH3)-interacting domain death agonist (BID) and executioner caspases. By contrast, executioner caspases cleave hundreds of different substrates and are largely responsible for the phenotypic changes seen during apoptosis. Initiator caspase activation first involves dimerization of inactive caspase monomers (see the figure, part **b**). In the case of caspase 8, following death receptor ligation, dimers are formed by the recruitment of caspase 8 monomers through their pro-domains to the adaptor molecule FAS-associated death domain protein (FADD). Dimerization and interdomain cleavage are required for the activation and stabilization of mature caspase 8 (REFS 131–133). Although dimerization is required for caspase 9 activation and interdomain cleavage occurs, cleavage is involved in the attenuation rather than promotion of caspase 9 activity[89,134]. The activation mechanism of executioner caspases differs from that of initiator caspases (see the figure, part **b**). Executioner caspases are present as dimers in cells and are activated by cleavage, leading to intramolecular rearrangements and the formation of an enzymatically active dimer.

causes hepatocyte apoptosis that leads to rapid, fatal hepatitis, whereas BID-deficient mice are resistant to this[3]. Combined loss of BID and XIAP restores hepatocyte apoptotic sensitivity and hepatitis following FASL injection, providing genetic proof that MOMP-induced XIAP antagonism is required for FASL-induced apoptosis in hepatocytes[4].

In vertebrates, most apoptotic stimuli require MOMP for caspase activation and apoptosis. In contrast, MOMP is dispensable for apoptosis in the invertebrate organisms *Drosophila melanogaster* and *Caenorhabditis elegans*[5]. When MOMP has been detected in *D. melanogaster*, it seems to be a consequence rather than a cause of caspase activation[6]. Interestingly, although MOMP does not contribute to apoptosis, fission of the tubular mitochondrial network promotes apoptosis in both *C. elegans* and *D. melanogaster* through an as yet undefined mechanism[6,7].

Tubular mitochondrial network
Multiple fused mitochondria forming filamentous, elongated structures. These networks are highly dynamic owing to constant rounds of mitochondrial fission and fusion.

In most cases, MOMP is a point of no return for cell survival as cells die irrespective of caspase activity following MOMP[8]. Given this importance, addressing how the mitochondrial outer membrane is selectively breached, and why this causes cell death, remains an intense area of basic and translational research. Here, we focus on recent studies that provide new insight into how MOMP occurs, the nature of membrane permeabilization and how the release of IMS proteins can be regulated post-MOMP. We then discuss the regulation of caspase activity post-MOMP and how MOMP brings about cell death in either a caspase-dependent or caspase-independent manner. Finally, we review data showing that MOMP is not always an obligatory death sentence, as some cells can recover.

Pulling the trigger: activation of MOMP

BCL-2-mediated regulation of MOMP is discussed only briefly here, and the reader is referred to recent, extensive reviews for further details[9,10]. Activation of either BCL-2-associated X protein (BAX) or BCL-2 antagonist or killer (BAK) is essential for MOMP as cells lacking both proteins fail to undergo MOMP and apoptosis in response to diverse intrinsic stimuli[11]. BAX and BAK activity is largely controlled through interactions with other members of the BCL-2 family (BOX 3).

On activation, BAX and BAK undergo extensive conformational changes, leading to the mitochondrial targeting of BAX and the homo-oligomerization of BAK and BAX[12–14]. Oligomerization of BAX or BAK is likely to be required for MOMP as mutants of either protein that fail to form oligomers are unable to cause MOMP[15,16]. FRET-based analysis of BAX-mediated liposome permeabilization has provided compelling, real-time evidence for direct and dynamic interactions between truncated BID (tBID) and BAX, which precede BAX membrane insertion and liposome permeabilization[17]. This supports a model in which BAX (and by analogy BAK) activation requires interaction with BH3-only proteins. Structural analysis of BAX in complex with a chemically stapled BCL-2-interacting mediator of cell death (BIM; also known as BCL2L11) BH3 domain peptide termed BIM SAHB (stablized α-helices of BCL-2 domains) revealed a somewhat unexpected interaction site[18]. BIM SAHB does not bind in the BAX hydrophobic BH3-binding pocket (as occurs when the BID BH3 domain binds BAK[19]) but, instead, binds on the opposite side of BAX. Mutations in BAX that inhibit BIM SAHB binding attenuate BAX-induced MOMP, supporting a functional relevance for this interaction during BAX activation. However, it remains unclear whether direct activator proteins such as tBID and BIM interact with BAX in a similar manner to BIM SAHB, and whether BAK undergoes a similar activation mechanism by BIM SAHB.

A model for BAK activation and oligomerization, supported by biochemical data, has recently been proposed[16] (FIG. 2). In this model, BAK activation leads to exposure of its BH3 domain and its insertion into the hydrophobic groove of an adjacent, activated BAK molecule. The interaction is reciprocated, leading to the formation of a symmetrical BAK homodimer. Higher-order BAK oligomers are formed by dimer–dimer interactions mediated

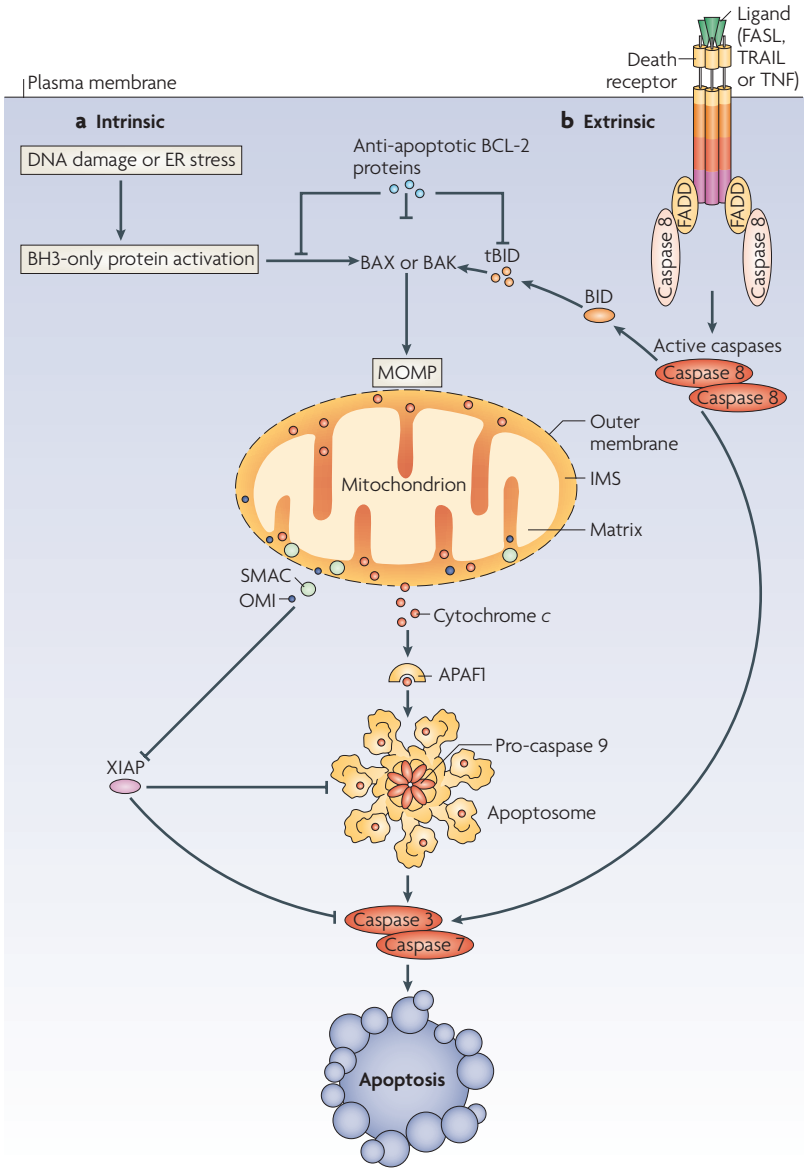


Figure 1 | **Intrinsic and extrinsic pathways of apoptosis. a** | Intrinsic apoptotic stimuli, such as DNA damage or endoplasmic reticulum (ER) stress, activate B cell lymphoma 2 (BCL-2) homology 3 (BH3)-only proteins leading to BCL-2-associated X protein (BAX) and BCL-2 antagonist or killer (BAK) activation and mitochondrial outer membrane permeabilization (MOMP). Anti-apoptotic BCL-2 proteins prevent MOMP by binding BH3-only proteins and activated BAX or BAK. Following MOMP, release of various proteins from the mitochondrial intermembrane space (IMS) promotes caspase activation and apoptosis. Cytochrome *c* binds apoptotic protease-activating factor 1 (APAF1), inducing its oligomerization and thereby forming a structure termed the apoptosome that recruits and activates an initiator caspase, caspase 9. Caspase 9 cleaves and activates executioner caspases, caspase 3 and caspase 7, leading to apoptosis. Mitochondrial release of second mitochondria-derived activator of caspase (SMAC; also known as DIABLO) and OMI (also known as HTRA2) neutralizes the caspase inhibitory function of X-linked inhibitor of apoptosis protein (XIAP). **b** | The extrinsic apoptotic pathway is initiated by the ligation of death receptors with their cognate ligands, leading to the recruitment of adaptor molecules such as FAS-associated death domain protein (FADD) and then caspase 8. This results in the dimerization and activation of caspase 8, which can then directly cleave and activate caspase 3 and caspase 7, leading to apoptosis. Crosstalk between the extrinsic and intrinsic pathways occurs through caspase 8 cleavage and activation of the BH3-only protein BH3-interacting domain death agonist (BID), the product of which (truncated BID; tBID) is required in some cell types for death receptor-induced apoptosis. FASL, FAS ligand; TNF, tumour necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand.

through a cryptic interface that is exposed following BAK activation[20]. Mutational analysis suggests that there is a similar mechanism for BAX homo-oligomerization[15].

Exactly how many molecules of BAX or BAK must oligomerize for MOMP to occur is unclear. One study found that four BAX molecules are sufficient to permeabilize an artificial membrane, whereas another study detected much larger BAX oligomers in apoptotic cells[21,22]. More recently, using single-cell imaging, the number of BAX molecules in a MOMP-inducing complex has been estimated at more than one hundred, although smaller complexes could not have been detected by this means owing to the limits of optical resolution[23]. Accurately determining the amount of active BAX and BAK molecules required for MOMP will provide insight into how MOMP occurs. However, this remains challenging owing largely to the small amounts of activated BAX and BAK that are required for MOMP and because both proteins continue to homo-oligomerize post-MOMP[24].

Mechanisms of MOMP

Although the requirement for activated BAX and BAK to induce MOMP is not debated, the means by which they carry out this task is. Here, we discuss the biophysical characteristics of MOMP, its kinetics and whether it allows for selective or non-selective release of IMS proteins. We then review how active BAX and BAK are thought to permeabilize the mitochondrial outer membrane.

Biophysical characteristics of MOMP. Live-cell imaging of MOMP, by monitoring the release of green fluorescent protein (GFP)–cytochrome *c* from mitochondria during apoptosis, demonstrated that, although the onset of MOMP is highly variable, most mitochondria undergo MOMP within 5 minutes of initiation[25]. More recently, single-cell imaging at high temporal resolution has shown that MOMP can initiate from a defined point or points in a cell and proceed in a wave-like manner across all mitochondria in the cell. Although the mechanism remains unclear, inhibitor studies implicate roles for protein phosphorylation and endoplasmic reticulum Ca^{2+} pumps in wave propagation[26–28]. Potentially, caspase activity may contribute to intracellular MOMP waves through cleavage and activation of BID. However, somewhat at odds with these findings is the observation that the length of time between the first and last mitochondrion in a cell to undergo MOMP is unaffected by lowering temperature, which argues against an enzymatic component to the process[25]. This discordance may be due to the higher temporal resolution achieved in more recent studies, or, perhaps rather than contributing to wave propagation, enzymatic processes may lower the initial threshold for individual mitochondria to undergo MOMP, thereby indirectly affecting release kinetics.

Whether MOMP displays any selectivity for the release of different IMS proteins has been subject to much scrutiny. BAX-mediated liposome permeabilization *in vitro* leads to the equally efficient release of 10 kDa and 2 MDa dextrans, suggesting that MOMP displays no selectivity for protein size[29]. In cells, proteins larger than 100 kDa (the predicted size of soluble SMAC–GFP dimers) are released following MOMP. In contrast, a tetrameric SMAC–dsRed

Box 2 | **Apoptogenic IMS proteins**

Cytochrome *c*.
Although cytochrome *c* is primarily recognized as a key component of electron transport during oxidative phosphorylation, it is also absolutely required for caspase activation following mitochondrial outer membrane permeabilization (MOMP). Cells lacking cytochrome *c* fail to activate caspases and are resistant to intrinsic apoptosis[135]. Moreover, knock-in mice expressing cytochrome *c* Lys27Ala, which retains respiratory chain function but cannot bind apoptotic protease-activating factor 1 (APAF1), display similar neurological phenotypes to APAF1- and caspase 9-null mice, and cytochrome *c* Lys72Ala knock-in cells fail to activate caspases and undergo apoptosis following pro-apoptotic stimuli[136]. Cytochrome *c* Lys72Ala-expressing thymocytes display apoptotic sensitivity, in contrast to the resistance seen in APAF1-null and BAX–BAK double knockout thymocytes[137], suggesting that other MOMP-dependent mediators of APAF1 activation might exist, although cytochrome *c* Lys72Ala might also retain a residual capacity to activate APAF1 (REF. 138).

SMAC and OMI
X-linked inhibitor of apoptosis protein (XIAP) inhibits caspase activity by directly binding active caspases, caspase 9, caspase 3 and caspase 7 (REF. 139). MOMP counteracts this through the release of second mitochondria-derived activator of caspase (SMAC; also known as DIABLO) and OMI (also known as HTRA2), two intermembrane space (IMS) proteins that directly bind XIAP and antagonize its ability to inhibit caspases[140–142]. Loss of SMAC or OMI, either alone or in combination, does not result in resistance to cell death. In fact, paradoxically, OMI-deficient cells are more sensitive to many intrinsic apoptotic stimuli, which may be due to the loss of OMI's mitochondria chaperone function[143]. The recent discovery that IAP antagonistic drugs induce degradation of IAPs, thereby deregulating nuclear factor κB (NF-κB) signalling and causing tumour necrosis factor (TNF)-dependent death, raises the interesting possibility that SMAC and OMI may also modulate IAP levels and NF-κB signalling following their release[144–146].

Others
The role of apoptosis-inducing factor (AIF) in promoting cell death is unclear. Following MOMP, AIF release is either slow or requires caspases and therefore probably does not contribute greatly to apoptotic cell death[31,147]. However, mitochondrial release of AIF (for example, by calpain[51]) may contribute to cell death in cell types such as neurons when caspase function downstream of MOMP is inhibited, leading to caspase-independent cell death (CICD). Endonuclease G is a mitochondrial IMS protein that can also be released following MOMP, whereby it contributes to apoptosis and CICD through cleavage of nuclear DNA[148]. However, endonuclease G deficiency has no effect on apoptotic DNA fragmentation or CICD[149].

fusion protein (with a predicted size of 190 kDa) failed to undergo release on MOMP, suggesting that MOMP may have size limitations *in vivo*[30]. Potentially, this retention may also be due to physicochemical properties such as enhanced membrane binding of the tetrameric molecule. Live single-cell imaging of multiple IMS proteins showed identical kinetics of IMS protein release from mitochondria following MOMP, irrespective of size[31]. However, in another study it was found that the duration of MOMP was slightly longer in the case of SMAC–mCherry (with a predicted dimeric size of 100 kDa) relative to cytochrome *c*–GFP (with a predicted size of 42 kDa)[28]. Importantly, in both studies the onset of SMAC and cytochrome *c* release was simultaneous, indicating that they exit mitochondria by a similar mechanism. Selective release of IMS proteins following MOMP has been seen in cells deficient for dystrophin-related protein 1 (DRP1; also known as UTRN), a dynamin-like protein that is required for mitochondrial fission. In DRP1-deficient cells, SMAC readily undergoes mitochondrial release but most cytochrome *c* is retained in mitochondria following MOMP[32–34]. However, the means by which DRP1 promotes mitochondrial cytochrome *c* release following MOMP remain unclear.

Proteinaceous channels. BCL-2 proteins such as BAX and BCL-XL (also known as BCL2L1) display structural similarities with bacterial pore-forming toxins, leading to the hypothesis that BAX and BAK themselves might directly form pores in the mitochondrial outer membrane[35,36] (FIG. 2). Along these lines, several studies have found that BAX forms ion channels or membrane pores in artificial membranes; however, anti-apoptotic BCL-2 proteins can also form membrane channels *in vitro*[37]. More recent evidence for MOMP occurring through a BAX or BAK pore has emerged from patch-clamping analysis of mitochondria undergoing MOMP. Using tBID to trigger MOMP, a mitochondrial outer membrane channel is formed that increases in conductance (and therefore size) over time, with similar kinetics to MOMP, implicating the channel (termed by the authors as the mitochondrial apoptosis-induced channel (MAC)) as the cause of MOMP[38]. The step-wise growth of these channels suggests that sequential recruitment of activated, membrane-bound BAX and BAK dimers to a small pore results in a channel of increasing size, which ultimately allows cytochrome *c* to be released from the mitochondrial IMS. Inhibitors that block MAC formation *in vitro* inhibit MOMP and apoptosis in cells, in support of MAC as the relevant MOMP-inducing mechanism, although it remains possible that these inhibitors may directly block BAX and BAK activation[39]. One caveat concerning the BAX or BAK pore model is that most studies have described channels that are only large enough to accommodate cytochrome *c*, but MOMP clearly allows for the release of much larger proteins, as noted above.

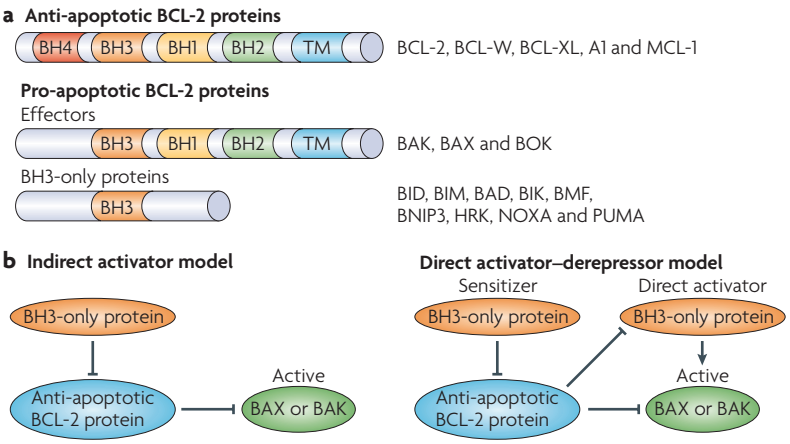
Rather than BAX and BAK forming pores themselves, it has been proposed that the modulation of existing mitochondrial channels such as the mitochondrial permeability transition pore complex (PTPC) — a multiprotein complex built up at the contact site between the inner and outer mitochondrial membranes — may have a causal role in MOMP. Cells lacking cyclophilin D, an essential component of the PTPC, display normal apoptotic sensitivity to a range of stimuli, effectively ruling out any role for the PTPC in MOMP[40–42]. Alternatively, MOMP has been postulated to require BAK and BAX modulation of voltage-dependent anion channel (VDAC) function. VDACs are the main pathway for metabolite diffusion across the mitochondria. However, loss of all three VDAC isoforms imparts no resistance of cells or isolated mitochondria to either MOMP or apoptosis[43]. Other studies have found that VDAC2 interactions with BAK are required to hold BAK inactive or for its mitochondrial localization[44,45].

Lipidic pores. An alternative model suggests that interaction of activated BAX and BAK with outer membrane lipids leads to membrane bending and, ultimately, formation of transient lipid pores or inverted micelles, thereby allowing IMS protein release[46–48] (FIG. 2). Lipid pores would account for several key aspects of MOMP, including the release of large IMS proteins and the difficulties in visualizing proteinaceous pores in the mitochondrial outer membrane. Accordingly, activated BAX can induce liposome permeabilization *in vitro*, leading to the release of encapsulated protein or dextrans in a size-independent manner[17,29]. Recently, cryo-electron microscopy (EM)

FRET
(Förster resonance energy transfer). The non-radiative transfer of energy from a donor fluorophore to an acceptor fluorophore that is typically <80 Å away. FRET will only occur between fluorophores in which the emission spectrum of the donor has a significant overlap with the excitation of the acceptor.

Liposome
A vesicle made of lipid bilayer in an aqueous environment. Membrane proteins can be incorporated in the bilayer.

Box 3 | **Regulation of MOMP by the BCL-2 family**



The B cell lymphoma 2 (BCL-2) family of proteins is divided into three groups based on their BCL-2 homology (BH) domain organization (see the figure, part **a**). Pro-apoptotic BCL-2 proteins can be sub-divided into effectors (the proteins that actually cause mitochondrial outer membrane permeabilization (MOMP)) or BH3 only (the proteins that relay the apoptotic signal to the effectors). Although BCL-2-related ovarian killer protein (BOK) displays similar domain architecture to BCL-2-associated X protein (BAX) and BCL-2 antagonist or killer (BAK), there is little evidence that it is a functional effector. Two prominent models of BAX and BAK activation have been proposed, termed the indirect activator (or neutralization) and direct activator–derepressor models of activation[150,151] (see the figure, part **b**). The indirect activator model asserts that BAX and BAK are bound in a constitutively active state by anti-apoptotic BCL-2 proteins and that competitive interactions of BH3-only proteins with anti-apoptotic BCL-2 family members is sufficient to release activated BAX and BAK. In the direct activator–derepressor model, BAX and BAK are activated following interaction with a subset of BH3-only proteins known as direct activators, and anti-apoptotic BCL-2 proteins prevent MOMP either by sequestering the activating BH3-only proteins or by inhibiting activated BAX and BAK. A second subset of BH3-only proteins, termed sensitizers, cannot directly activate BAX and BAK but neutralize anti-apoptotic BCL-2 proteins. Definitive proof for either model has proved challenging; it is likely that aspects of both models are correct. BAD, BCL-2 antagonist of cell death; BID, BH3-interacting domain death agonist; BIK, BCL-2-interacting killer; BIM, BCL-2-interacting mediator of cell death; BMF, BCL-2-modifying factor; BNIP3, BCL-2 and adenovirus E1B 19 kDa protein-interacting protein 3; HRK, harakiri; PUMA, p53 upregulated modulator of apoptosis; TM, transmembrane.

analysis of BAX-permeabilized liposomes revealed openings of varying size (25–100 nm) that appeared concurrently with permeabilization in a manner that was BCL-XL inhibitable[49]. The diameter of these openings is consistent with the ability of BAX to induce the size-independent release of dextrans. Supporting a lipidic pore model, the edges of these BAX-induced pores are smooth and devoid of proteinaceous material. In contrast, protein pores formed by the toxin pneumolysin, as analysed by cryo-EM, are uniform in nature and decorated around the edges with toxin molecules[50]. However, similar pore-like structures have yet to be found on mitochondria during MOMP.

Patch clamping
An electrophysiological technique used for measuring ion channel activity over membranes. Typically, a small diameter (1 μm) micropipette serves as the electrode and is applied to a small area of membrane (the 'patch'), allowing the activity of one or a few ion channels to be measured.

Micelle
An aggregate (typically spherical) of varying size comprised of lipids. In aqueous environments, the hydrophobic lipid tails orientate to the centre of the micelle and the hydrophilic head groups are on the surface.

Post-MOMP regulation of IMS proteins

Although MOMP itself provides little specificity as to which IMS proteins are released, studies suggest that release of different IMS proteins can be selective. This may be through the regulation of IMS protein interaction with mitochondrial membranes or by mitochondrial inner membrane remodelling.

IMS protein membrane attachment. Apoptosis inducing factor (AIF) is an IMS protein that is anchored on the mitochondrial inner membrane and displays much slower release kinetics than cytochrome *c* following MOMP[31]. This protracted release may be owing to the requirement for AIF cleavage post-MOMP by cytosolic proteases to liberate AIF from the mitochondrial inner membrane. One candidate protease is cytosolic calpain I, which can cleave AIF *in vitro* to promote its release from permeabilized mitochondria[51].

Electrostatic interactions between cytochrome *c* and the mitochondrial lipid cardiolipin have been proposed to regulate its release[52]. However, it might be expected that the ionic strength of the cytosol should suffice to disrupt these interactions[53].

Post-MOMP mitochondrial inner membrane remodelling. Other mechanisms may control IMS protein release following MOMP. Mitochondrial cristae are involutions of the mitochondrial inner membrane that greatly increase the mitochondrial surface area available for oxidative phosphorylation and ATP synthesis. Cristae are dynamic structures and their accessibility to the IMS is largely dictated through regulation of cristae junction size. As most cytochrome *c* resides in mitochondrial cristae, several studies have addressed whether cristae remodelling provides an additional means of regulating cytochrome *c* release following MOMP. Various BH3-only proteins, including BID, BIM, BNIP3 (BCL-2 and adenovirus E1B 19 kDa protein-interacting protein 3) and BCL-2-interacting killer (BIK) have been found to promote mitochondrial cristae remodelling[54–57]. Treatment of mitochondria *in vitro* with the BH3 protein tBID induced dramatic inner membrane remodelling, leading to interconnected cristae with widened junctions and cytochrome *c* mobilization into the IMS[55]. Two IMS proteins, optic atrophy protein 1 (OPA1; a dynamin-like GTPase) and presenilins-associated rhomboid-like protein (PARL; a rhomboid protease) have been found to regulate cristae remodelling during apoptosis[58,59]. Following MOMP, disassembly of OPA1 hetero-oligomers is required for the widening of cristae junctions, whereas PARL cleavage of OPA1 generates an OPA1 cleavage product that maintains tight cristae junctions that prevent cytochrome *c* release. Functionally, PARL loss renders cells more susceptible to apoptosis induced by intrinsic stimuli, whereas OPA1 overexpression is protective[58,59].

Although cristae remodelling requires activated BAX or BAK, it can occur in the absence of MOMP because pharmacological inhibitors of MOMP still allow remodelling to occur[54]. Remodelling was associated with the mobilization of cytochrome *c* to the IMS and, like previous studies, disassembly of OPA1 was required for remodelling to occur. In this study, however, gross changes in mitochondrial morphology were not apparent. Instead, a subtle narrowing, rather than widening, of cristae junctions occurred. Similarly, correlative light microscopy and EM of apoptotic cells revealed that gross alterations in mitochondrial structure were detected only after MOMP and caspase activation had occurred, arguing against a causal role for large mitochondrial structural changes

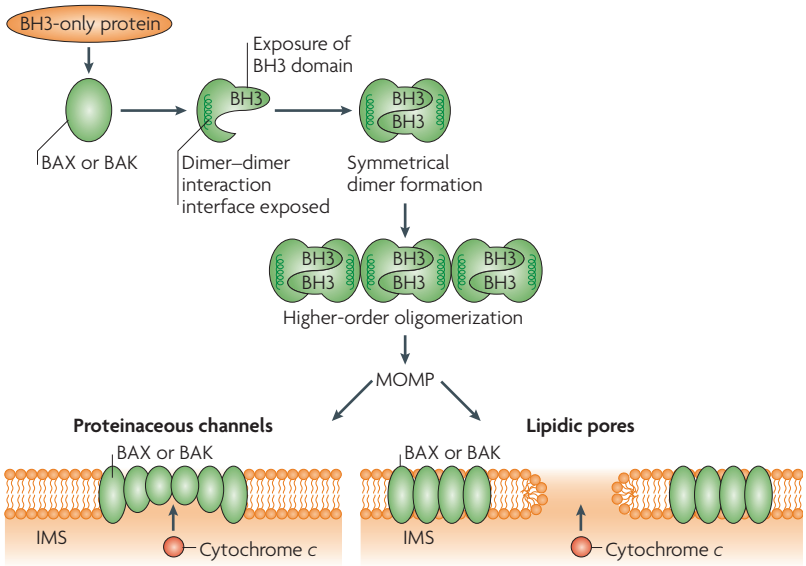


Figure 2 | **BAX and BAK activation and pore formation.** The binding of B cell lymphoma 2 (BCL-2) homology 3 domain (BH3)-only proteins to BCL-2-associated X protein (BAX) and BCL-2 antagonist or killer (BAK) leads to extensive conformational changes during their activation. The BH3 domain and hydrophobic cleft are exposed, allowing symmetrical BAX or BAK dimers to form through reciprocal BH3 domain–cleft interactions. During activation, a dimer–dimer interaction surface is also exposed, allowing higher-order oligomers to form. Higher-order oligomers promote mitochondrial outer membrane permeabilization (MOMP) by unclear means, perhaps through forming proteinaceous channels or by destabilizing lipid membranes and forming lipidic pores. IMS, intermembrane space.

in promoting IMS protein release[60]. Given that even in a closed conformation cristae junction width should easily accommodate cytochrome *c* exit, it is unlikely that alterations in cristae width *per se* regulate cytochrome *c* release. One study has found that cytochrome *c* resides either in the IMS or in the mitochondrial cristae and these pools do not readily interchange[61]. By contrast, the basal diffusability of cytochrome *c* in mitochondria has recently been shown to account for its rapid and complete release on MOMP[62]. Some studies have found that partial release of cytochrome *c* is sufficient to drive apoptosis, albeit at slower kinetics, whereas others have found apoptosis is blocked under these conditions[32–34,54,55]. These contrasting results may simply reflect varying thresholds for cytochrome *c*-induced caspase activation in different cell types[63].

Cristae junction
A connection between the mitochondrial cristae and the mitochondrial IMS. The diameter of cristae junctions can be altered, thereby regulating the accessibility of mitochondrial cristae to the IMS.

Nitrosylation
A post-translational protein modification involving the addition of a nitrosyl group to the Cys residue of a target protein, potentially altering target protein function.

Post-MOMP regulation of caspase activity

In addition to inhibition by XIAP, various mechanisms curtail caspase activity following MOMP. Under healthy conditions, these inhibitory mechanisms may exist to preserve cell viability should accidental MOMP occur in a limited number of mitochondria, but they are overwhelmed when MOMP occurs in most mitochondria, such as during apoptosis. Caspase activation following MOMP in a minority of mitochondria has been proposed to initiate a MOMP amplification loop through the executioner caspase-mediated cleavage and activation of proteins such as BID and BCL-2 (REFS 64,65). Post-MOMP regulation of caspase activity centres around the regulation of caspase 9 activity, either directly or indirectly, through effects that occur on cytochrome *c*-induced APAF1 apoptosome formation (FIG. 3).

Regulation of apoptosome assembly. Following mitochondrial release, cytochrome *c* promotes APAF1 conformational changes, leading to APAF1 oligomerization and assembly into a heptameric, wheel-like structure (the apoptosome) that recruits pro-caspase 9, promoting its dimerization and activation. Apoptosome formation requires APAF1-mediated dATP binding[66]. Paradoxically, physiological levels of nucleotides inhibit apoptosis by directly binding cytochrome *c*, preventing APAF1–cytochrome *c* interactions and apoptosome formation[67]. Along similar lines, transfer RNA (tRNA) binds cytochrome *c* and inhibits apoptosome formation by blocking the interaction of cytochrome *c* with APAF1 (REF. 68).

The pro-apoptotic activity of cytochrome *c* may also be regulated by redox, whereby cytochrome *c* oxidation promotes its pro-apoptotic activity and reduction inhibits it[69,70]. Mechanistically, the means by which the redox status affects the pro-apoptotic function of cytochrome *c* is unknown and other studies have found that reduced cytochrome *c* is still proficient at activating caspases *in vitro*[71,72]. The addition of a haem moiety to cytochrome *c* occurs in the mitochondrial IMS and is required for its ability to promote caspase activity following MOMP[73]. Interestingly, nitrosylation of the cytochrome *c* haem moiety occurs under apoptotic conditions[74]. Modelling the effects of nitrative stress by disruption of the cytochrome *c* Met80–haem interaction promotes cytochrome *c* nuclear translocation in non-apoptotic cells, leading to the upregulation of a protective stress response. This suggests that nitrosylation can impart novel non-apoptotic roles on cytochrome *c*[75].

Normal intracellular levels of potassium also inhibit apoptosome assembly. This inhibition can be overcome by increased concentrations of cytochrome *c*, suggesting that extensive MOMP, as seen during apoptosis, is required for caspase activation and apoptosis[76]. Intracellular levels of Ca^{2+} have also been found to inhibit apoptosome activity by blocking nucleotide exchange on monomeric APAF1, thereby inhibiting apoptosome formation[77]. Various proteins, including heat shock proteins such as HSP70 and HSP90, have been shown to negatively influence apoptosome function, either by inhibiting its formation or by preventing the recruitment of pro-caspase 9 (REFS 78–81).

Apoptosome activity can also be positively modulated, thereby enhancing caspase 9 activity. Putative HLADR-associated protein I (PHAPI; also known as pp32) stimulates apoptosome activity and caspase activation by preventing APAF1 aggregation and promoting nucleotide exchange on APAF1 (REFS 82,83). Reduced expression of PHAPI imparts apoptotic resistance to cells, enabling clonogenic survival that may be relevant during tumorigenesis[84]. Interestingly, APAF1 also has a non-apoptotic role in regulating DNA damage-induced cell cycle arrest, raising the possibility that modulators of APAF1 apoptotic function can also alter its cell cycle checkpoint functions[85].

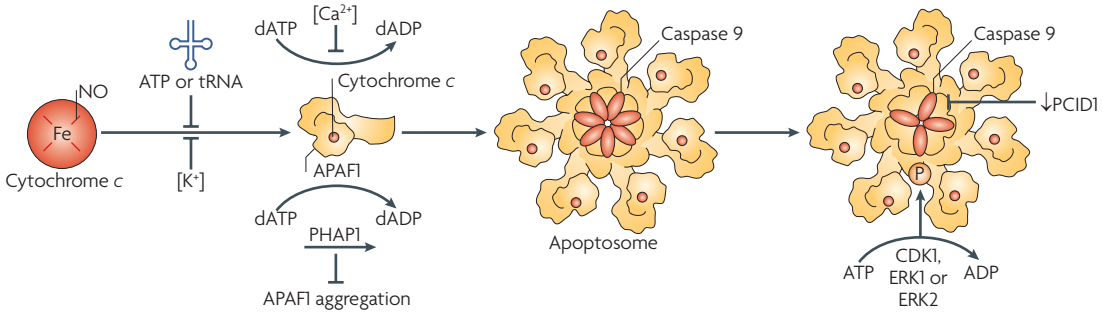


Figure 3 | **Post-MOMP regulation of caspase activity.** Cytochrome *c* binds an apoptotic protease-activating factor 1 (APAF1) monomer, leading to its oligomerization into a heptameric wheel-like structure called the apoptosome that recruits and activates caspase 9. Physiologic levels of nucleotides such as ATP or transfer RNA (tRNA) can block cytochrome *c* binding to APAF1 and inhibit apoptosome formation by directly binding cytochrome *c*. Cytochrome *c* requires its haem moiety, which is acquired in the mitochondrial intermembrane space, in order to bind and activate APAF1. Nitrosative stress can modify the haem moiety and attenuate the pro-apoptotic function of cytochrome *c*. Intracellular levels of potassium inhibit apoptosome formation, at least in part, by competing with cytochrome *c* for APAF1 binding, but high levels of cytochrome *c* can overcome this inhibition. Binding of cytochrome *c* to APAF1 stimulates APAF1-dependent dATP hydrolysis, driving apoptosome formation. Putative HLADR-associated protein I (PHAPI; also known as pp32) enhances this process by both promoting nucleotide exchange on APAF1 and inhibiting aggregation of the APAF1 monomer. Intracellular Ca^{2+} can inhibit nucleotide exchange, thereby blocking apoptosome formation. Direct phosphorylation of caspase 9 by kinases, including cyclin-dependent kinase 1 (CDK1)–cyclin B1, extracellular signal-regulated kinase 1 (ERK1; also known as MAPK3) and ERK2 (also known as MAPK1), inhibits caspase 9 activity by unknown means. Finally, downregulation of PCI domain-containing protein 1 (PCID1; also known as EIF3M) can negatively regulate caspase 9 levels, thereby effecting caspase activation following mitochondrial outer membrane permeabilization (MOMP). NO, nitric oxide.

Regulation of caspase 9 activation. Several kinases have been shown to phosphorylate caspase 9, inhibiting its enzymatic activity[86]. In human caspase 9, Thr125 is the main inhibitory phosphorylation site and is targeted by several kinases, including extracellular signal-regulated kinase 1 (ERK1; also known as MAPK3), ERK2 (also known as MAPK1) and cyclin-dependent kinase 1 (CDK1)–cyclin B1 (REFS 87,88). Phosphorylation of Thr125 or mutation to a phosphomimetic residue impairs the ability of cytochrome *c* to induce caspase activity *in vitro*[88]. During mitosis, CDK1–cyclin B1-mediated phosophorylation of caspase 9 on Thr125 attenuates its activity. Prolonged mitotic arrest, induced by microtubule stabilizing agents such as taxol, leads to caspase 9-dependent death that can be enhanced by inhibition of caspase 9 phosphorylation at Thr125 (REF. 87). Although it is clear that phosphorylation can negatively affect caspase 9 activity, the means by which this occurs is unclear as it does not affect the recruitment of caspase 9 to the apoptosome[88]. Whether phosphorylation inhibits other aspects of caspase 9 activation, such as its ability to dimerize, remains to be tested.

Apoptosome-mediated activation of caspase 9 leads to caspase 9 auto-processing, which greatly reduces its affinity for the apoptosome and results in the loss of caspase 9 activity[89]. Therefore, apoptosome-mediated caspase 9 activation is a 'molecular timer', the activity of which is largely due to intracellular caspase 9 levels. Accordingly, regulation of caspase 9 expression also controls caspase activity post-MOMP. PCI domain-containing protein 1 (PCID1; also known as EIF3M) is the human orthologue of Tango 7, a pro-apoptotic effector that regulates expression of the *D. melanogaster* initiator caspase pro-Dronc[90]. Interestingly, downregulation of PCID1 decreases expression of pro-caspase 9, leading to the reduction of caspase activity during apoptosis. Although it is not known how PCID1 regulates pro-caspase 9 levels, the finding that PCID1 is commonly downregulated in pancreatic cancer suggests that it may be clinically important[91].

The end game: how MOMP kills cells

MOMP leads to the rapid activation of caspases and apoptosis. However, in the absence of caspase activity (for example in APAF1- and caspase 9-deficient backgrounds[92–94]), cells undergo caspase-independent cell death (CICD), which thereby defines MOMP as a point of no return (see Supplementary information S2 (movie)). Although cell death is the usual outcome following MOMP, the mechanisms and kinetics by which cells die differ greatly depending on caspase activity. Here, we review what happens after MOMP and how these events bring about cellular demise through caspase-dependent and caspase-independent means (FIG. 4).

Cellular effects of MOMP. Following MOMP, caspase activation ensues and results in the cleavage of hundreds of proteins and, ultimately, apoptosis. Caspase cleavage of any given protein substrate can activate or inhibit its function and, although hundreds of caspase substrates have been identified, many are probably innocent bystanders that play no actual part in apoptosis[2]. In the absence of caspase activity, cell death normally occurs following MOMP, albeit with much slower kinetics than apoptosis. MOMP has been proposed to cause CICD either by the release of IMS proteins, such as AIF and endonuclease G, or through a progressive decline in mitochondrial function leading, among other effects, to ATP depletion[95]. Cells can continue to undergo cell division following MOMP provided that caspase activity is inhibited[96].

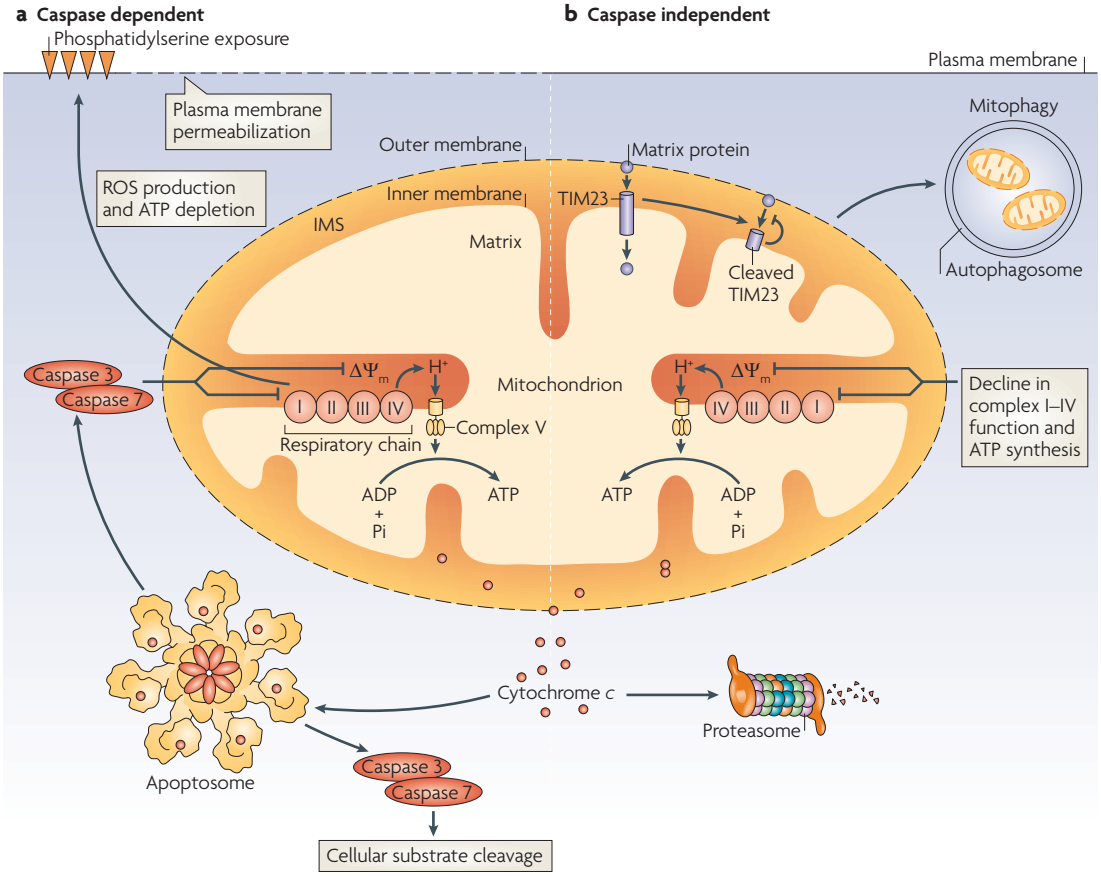


Figure 4 | **Cellular effects of MOMP. a** | Caspase-dependent effects. Mitochondrial outer membrane permeabilization (MOMP) leads to the release of cytochrome *c* from mitochondria, which activates caspases to cleave numerous cellular substrates, causing apoptosis. Respiratory chain complexes I–IV generate the proton gradient over the mitochondrial inner membrane that drives ATP generation by ATP synthase (complex V). Executioner caspases (caspase 3 and caspase 7) enter the mitochondrial intermembrane space (IMS) following MOMP, disrupting complex I and complex II activity. In the case of complex I, this occurs partly through cleavage of an essential complex I subunit, NADH–ubiquinone oxidoreductase 75 kDa subunit (NDUFS1). Collectively, these caspase-dependent effects lead to a loss of transmembrane potential ($\Delta\Psi_m$) and ATP synthesis, and an increase in reactive oxygen species (ROS) production. These effects of mitochondrial dysfunction contribute to the exposure of phosphatidylserine on the outer leaflet of the plasma membrane and its permeabilization, which occurs during apoptosis. **b** | Caspase-independent effects. Following MOMP, initial levels of cytochrome *c* in the cytoplasm are sufficient to support respiration in the permeabilized mitochondria. In the long-term, levels of cytochrome *c* might be rate limiting owing to proteasome-dependent degradation. Even in the absence of caspase activity, respiratory chain complex I – IV activity drops over time, leading to a gradual loss in $\Delta\Psi_m$ and ATP synthesis, which effectively starves the cell. TIM23 is an essential component of the inner membrane protein translocase. Following MOMP, TIM23 undergoes inactivation through cleavage by an unknown intramitochondrial protease, effectively blocking new protein import into the mitochondrial matrix. Finally, MOMP triggers the removal of permeabilized mitochondria by the autophagic machinery, a process termed mitophagy.

In this study, CICD correlated with a progressive decline in the mitochondrial function and ATP generation that preceded the mitochondrial release of AIF and endonuclease G, suggesting that MOMP contributes to CICD primarily through loss of mitochondrial function.

Mitochondrial effects of MOMP. Mitochondria are dynamic organelles that constantly undergo cycles of fission and fusion with one another. Wide-scale mitochondrial fission occurs at or around the point of MOMP, irrespective of caspase activity but dependent on DRP1 (REF. 97). The role of mitochondrial fission during apoptosis in mammalian cells is unclear and, although fission occurs after MOMP, it may not depend on it[28,98,99]. MOMP and fission can be dissociated as fission occurs in situations where MOMP is blocked following an apoptotic stimulus, for example when BCL-XL is overexpressed[98]. Indeed, other studies have found that BCL-2 family members regulate mitochondrial morphology in healthy cells[100,101].

Although fission is not required for MOMP, DRP1 may contribute to this process. Pharmacological inhibitors of DRP1 block MOMP *in vitro*, a setting in which mitochondrial fission does not occur, thereby implying that DRP1 contributes to BAX- or BAK-induced MOMP independently of mitochondrial fission[102]. However, cells lacking DRP1 undergo MOMP, ruling out an absolute requirement for DRP1 for this event[33,103].

The maintenance of mitochondrial transmembrane potential ($\Delta\Psi_m$) is crucial for many mitochondrial functions, including ATP synthesis, ion homeostasis and protein import into the mitochondrial matrix. Following MOMP, $\Delta\Psi_m$ is dissipated through caspase-dependent and caspase-independent means[104,105]. Caspase-dependent dissipation of $\Delta\Psi_m$ is mediated, at least in part, through caspase cleavage of NADH–ubiquinone oxidoreductase 75 kDa subunit (NDUFS1), an essential component of respiratory chain complex I[106]. In permeabilized mitochondria, executioner caspases gain entry to the IMS and cleave NDUFS1, which leads to a sequential reduction in complex I activity, a drop in $\Delta\Psi_m$, a rapid reduction in ATP synthesis and an increase in reactive oxygen species (ROS). Interestingly, expression of a non-cleavable form of NDUFS1 delays the kinetics of $\Delta\Psi_m$ loss and phosphatidylserine exposure on the outer leaflet of the plasma membrane (a key feature of apoptosis that contributes to the phagocytosis of the dying cell) following MOMP. This indicates a direct role for caspase-induced mitochondrial dysfunction in mediating this process. Furthermore, modulation of mitochondrial dysfunction during apoptosis by the expression of non-cleavable NDUFS1 alters how the immune system responds to a dying cell[107]. Caspase-dependent mitochondrial ROS production oxidizes the immunostimulatory protein high mobility group protein B1 (HMGB1) in dying cells, thereby promoting immune tolerance, whereas expression of non-cleavable NDUFS1 reduces ROS levels, which blocks HMGB1 oxidation and leads instead to an immunostimulatory response. There are probably other mitochondrial caspase targets as non-cleavable NDUFS1 only partially rescues the caspase-dependent loss in $\Delta\Psi_m$, and respiratory chain complex II activity is also inhibited following MOMP in a caspase-dependent manner[106,108].

Mitochondrial function deteriorates even in the absence of caspase activity, leading to a progressive loss in $\Delta\Psi_m$ and ATP production, although how this occurs remains unclear[96,109]. Analysis of cells undergoing CICD has shown that respiratory complexes I and IV are lost in the absence of caspase activity at later time points following MOMP. One obvious reason for the loss of respiratory function might be cytochrome *c* release. However, following MOMP, cytochrome *c* remains at sufficiently high levels in the mitochondrial IMS to allow respiration[105]. At later time points, proteasome-dependent degradation of cytochrome *c* may promote respiratory dysfunction[110]. Alternatively, access of cytosolic enzymes to the mitochondrial IMS following MOMP may lead to inactivating post-translational modifications such as cleavage of crucial mitochondrial proteins. TIM23, an essential component of the inner membrane protein translocase complex, undergoes proteolytic inactivation following MOMP[111]. Cleavage of TIM23 was found to require an intramitochondrial protease and was associated with reduced cell viability following MOMP. Dysfunctional mitochondria can be specifically targeted for autophagic degradation through a process termed mitophagy. Mitophagy triggers include the loss of mitochondrial membrane potential and membrane permeability transition[112–114]. Interestingly, MOMP has also been shown to promote mitophagy[109,115]. Although mitophagy is primarily a homeostatic mechanism to ensure damaged mitochondria are removed, taken to its extreme, mitophagy can remove all mitochondria from a cell, effectively committing that cell to death[115]. However, one recent study showed that complete removal of mitochondria by enhanced mitophagy did not result in cell death for at least 4 days, suggesting that this is not a mechanism of cell death in short time frames[114]. The finding that cells can survive, at least in the short term, without mitochondria also suggests that CICD is not due solely to the loss of mitochondrial function, but may also involve an active role for permeabilized mitochondria.

Cellular recovery post-MOMP

The prevailing view that MOMP is a point of no return for cell survival is likely to be true in most, but importantly not all, situations. Recovery from MOMP probably has important pathophysiological consequences, enabling long-term survival of post-mitotic cells and promoting tumour cell survival[95]. Here, we review the mechanisms that govern cell survival following MOMP.

Survival following 'accidental' MOMP. Typically, MOMP causes the permeabilization of most mitochondria, leading to lethal caspase activation. However, studies have shown that there is a threshold for cytochrome *c*-mediated caspase activation that is influenced by many factors such as nucleotide and XIAP levels, as we have already discussed. This raises the possibility that in a minority of mitochondria MOMP might be insufficient to trigger apoptosis. Laser irradiation of neuronal mitochondria, leading to permeabilization of 15% of the mitochondrial population, was insufficient to trigger apoptosis[116]. However, whether accidental MOMP occurs in a few mitochondria in the absence of apoptosis remains an open question.

Post-mitotic cellular recovery. Sympathetic neurons and cardiomyocytes can survive following MOMP, perhaps necessitated by the life-long requirement for these post-mitotic cells[117,118]. Following terminal differentiation, both cell types express low levels of APAF1 and are unresponsive to microinjection of cytochrome *c*[119,120]. Apoptotic sensitivity is restored following upregulation of APAF1, addition of recombinant SMAC or deletion of XIAP, implicating an important role for XIAP-mediated caspase inhibition in regulating cell death. Both cell types survive following MOMP, suggesting that endogenous SMAC and OMI are at insufficient levels to neutralize XIAP activity[121,122]. In the case of neurons, prolonged apoptotic signalling following nerve growth factor (NGF) withdrawal induces a so-called 'competence to die' owing to down-regulation of XIAP levels[121]. The redox status of cytochrome *c* influences its pro-apoptotic activity following MOMP in neurons[123] (see above). Neurons display high levels of glycolysis, which, besides producing ATP, raises the intracellular levels of glutathione synthase (GSH) though the pentose–phosphate shunt. Following MOMP, cytochrome *c* is reduced and held inactive by GSH, which inhibits caspase activation, whereas oxidation of cytochrome *c* promotes its activity. Tumour cells, similar to

Transmembrane potential
The voltage (or electrical potential) difference between one side of a membrane and the other.

Post-mitotic cell
A cell that is neither preparing to nor undergoing cell division.

Pentose–phosphate shunt
A metabolic pathway that generates NADPH and pentose sugars from glucose-6-phosphate. Indirectly, NADPH serves as an important antioxidant by reducing glutathione.

neuronal cells, are typically glycolytic and therefore may also inhibit the pro-apoptotic activity of cytochrome *c* in a similar manner.

Recovery in proliferating cells. Proliferating cells can also recover from MOMP provided caspase activity is inhibited. This may have important implications for oncogenesis and cancer therapy because tumour cells often display defects in caspase activation downstream of MOMP, through diverse mechanisms such as reduction in APAF1 expression or upregulation of XIAP[109,124,125]. Using a retroviral cDNA screen, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was found to protect cells from death downstream of MOMP, provided that caspase activity was inhibited, owing to its well-characterized role in glycolysis and a novel role in autophagy induction, partly through the upregulation of autophagy-related protein 12 (ATG12)[109]. The anticancer drug imatinib (Gleevec; Novartis), inhibits breakpoint cluster region protein (BCR)–abelson (ABL) kinase function and promotes both apoptosis and CICD. Interestingly, in some imatinib-resistant BCR–ABL-expressing cells, higher levels of GAPDH produce resistance to CICD that can be reverted by limited small interfering RNA knockdown of GAPDH, suggesting that protection from CICD by GAPDH may be therapeutically relevant[126].

One intriguing aspect concerning cellular recovery following MOMP is how the crucial process of cellular repopulation with intact mitochondria occurs. Addressing this issue, a recent study has found that MOMP can be incomplete, such that some mitochondria fail to undergo MOMP following an apoptotic stimulus and remain intact[127]. Increased levels of anti-apoptotic BCL-2 proteins on specific mitochondria probably account for their resistance to MOMP, supported by a lack of BAX or BAK activation on these mitochondria and reversion to complete MOMP after treatment with the BCL-2 antagonist, ABT-737. Importantly, the presence of intact mitochondria strongly correlates with cellular recovery under conditions of MOMP, suggesting that these are the 'seed' mitochondria that can repopulate the cell. In healthy neurons, MOMP leading to caspase 3 activation is required for effective AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor internalization at postsynaptic junctions[128]. Incomplete MOMP probably plays an important role in this process by promoting receptor internalization while preserving cell viability.

Concluding remarks

Considerable progress has been made in recent years addressing the regulation of MOMP, how it occurs and why it brings about cell death. However, many outstanding questions remain. Although we are beginning to understand how BAX and BAK become activated, the means by which they permeabilize the mitochondrial outer membrane remain elusive. Cell survival is possible following MOMP but it remains unclear whether MOMP in a minority of mitochondria actually occurs under healthy conditions, an event that would explain why cells can regulate caspase activity post-MOMP.

How MOMP initiates caspase activation leading to apoptosis is relatively well elucidated. In contrast, beyond 'mitochondrial catastrophe', we have very little mechanistic insight into how MOMP contributes to cell death in a caspase-independent manner. Following from this, the manner by which a cell dies following MOMP, either by caspase-dependent or caspase-independent means, may have profound effects on how the immune system and neighbouring cells react to it, but this is under-studied. Finally, as we have discussed, MOMP need not always lead to a dead end: both post-mitotic and mitotic cells can recover from MOMP, although the detailed mechanisms governing cell survival remain scarce. In summary, many fundamental questions remain about the process of MOMP and how it controls life and death, and we anticipate that future findings will greatly facilitate the manipulation of this process for therapeutic purposes.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES

UniProtKB: http://www.uniprot.org
AIF | APAF1 | BAK | BAX | BCL-XL | BID | BIK | BIM | BNIP3 | caspase 3 | caspase 7 | caspase 8 | caspase 9 | DRP1 | FADD | FASL | NDUFS1 | OMI | OPA1 | PARL | PCID1 | PHAPI | SMAC | TIM23 | XIAP

FURTHER INFORMATION

Douglas R. Green's homepage: http://www.stjude.org/green
Deathbase: http://deathbase.org/

SUPPLEMENTARY INFORMATION

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