Mitochondrial Regulation of Cell Death

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Although required for life, paradoxically, mitochondria are often essential for initiating apoptotic cell death. Mitochondria regulate caspase activation and cell death through an event termed mitochondrial outer membrane permeabilization (MOMP); this leads to the release of various mitochondrial intermembrane space proteins that activate caspases, resulting in apoptosis. MOMP is often considered a point of no return because it typically leads to cell death, even in the absence of caspase activity. Because of this pivotal role in deciding cell fate, deregulation of MOMP impacts on many diseases and represents a fruitful site for therapeutic intervention. Here we discuss the mechanisms underlying mitochondrial permeabilization and how this key event leads to cell death through caspase-dependent and -independent means. We then proceed to explore how the release of mitochondrial proteins may be regulated following MOMP. Finally, we discuss mechanisms that enable cells sometimes to survive MOMP, allowing them, in essence, to return from the point of no return.

In most organisms, mitochondria play an essential role in activating caspase proteases through a pathway termed the mitochondrial or intrinsic pathway of apoptosis. Mitochondria regulate caspase activation by a process called mitochondrial outer membrane permeabilization (MOMP). Selective permeabilization of the mitochondrial outer membrane releases intermembrane space (IMS) proteins that drive robust caspase activity leading to rapid cell death. However, even in the absence of caspase activity, MOMP typically commits a cell to death and is therefore considered a point of no return (Fig. 1). Because of this pivotal role in dictating cell fate, MOMP is highly regulated, mainly through interactions between pro- and anti-apoptotic members of the Bcl-2 family. In this article, we begin by discussing how mitochondria may have evolved to become central players in apoptotic cell death. We then provide an overview of current models addressing the mechanics of MOMP, outlining how this crucial event leads to cell death through both caspase-dependent or -independent mechanisms. Finally, we discuss how caspase activity may be regulated post-MOMP and define other processes that allow cells to survive MOMP and, in effect, return from the point of no return.

MITOCHONDRIA—NATURAL-BORN KILLERS?

The endosymbiosis theory of evolution posits that mitochondria are modern-day descendants

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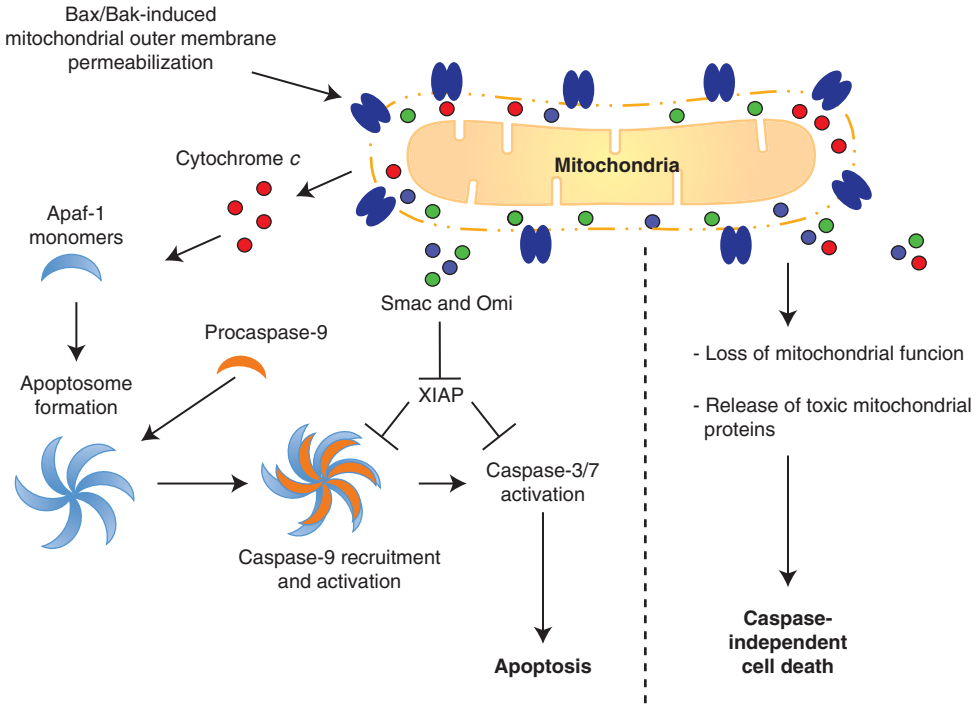

Figure 1. Mitochondrial regulation of cell death. Bax/Bak-mediated mitochondrial outer membrane permeabilization (MOMP) can lead to caspase-dependent apoptosis (*left*) or caspase-independent cell death (*right*). Following MOMP, soluble proteins are released from the mitochondrial intermembrane space into the cytoplasm. Cytochrome *c* binds to monomeric Apaf-1 leading to its conformational change and oligomerization. Procaspase-9 is recruited to heptameric Apaf-1 complexes forming the apoptosome. This leads to activation of caspase-9 and, through caspase-9-mediated cleavage, activation of the executioner caspases-3 and -7. Release of Smac and Omi from the mitochondrial intermembrane space facilitates caspase activation by neutralizing the caspase inhibitor XIAP. MOMP can also lead to nonapoptotic cell death through a gradual loss of mitochondrial function and/or release of mitochondrial proteins that kill the cell in a caspase-independent manner.

of α-proteobacteria that invaded archeon cells more than 2 billion years ago (Gray 2012). This invasion, ultimately forming the original eukaryotic cell, may have simultaneously forged a role for mitochondria in cell death. One possibility is that, following bacterial invasion, the archeon underwent altruistic cell death in order to protect the clonal population (James and Green 2002; Green 2011). Over time, some bacteria may have been able to prevent cell death, forming an endosymbiotic relationship with the archeon and eventually giving rise to mitochondria as we know them today. It may be that Bcl-2 proteins are modern-day descendants of toxins expressed by bacteria to kill one another that were initially co-opted to enable permeabilization of the mitochondrial outer membrane (which is likely host cell-derived, based on composition) while sparing the mitochondrial inner membrane (which resembles bacterial membrane composition). Accordingly, Bcl-2 proteins display structural similarities to certain bacterial toxins including diphtheria toxin β-chain and the colicins (Muchmore et al. 1996; Suzuki et al. 2000). Over time, as with most mitochondrial functions, genetic control of the proteins that regulate cell death may have transferred to the nucleus, whereas the mitochondrial outer membrane remains the battlefield.

Mitochondria play a role in apoptosis in most animals; however, the extent and importance of their contribution differs greatly be-

tween organisms (Oberst et al. 2008). In mammals, the essential requirement for MOMP as an initiating event in caspase activation and apoptosis is best evidenced in mice lacking Bax and Bak (Lindsten et al. 2000; Wei et al. 2001). Cells derived from these mice are profoundly resistant to all intrinsic apoptotic stimuli, and Bax/Bak double-knockout mice display developmental defects consistent with inhibition of cell death. In stark contrast, in the nematode *Caenorhabditis elegans* or the fly *Drosophila melanogaster*, two organisms that have been used extensively in cell death research, mitochondria do not appear to play a major role in the activation and execution of apoptosis. In *Caenorhabditis elegans*, although the proteins that control caspase activation are located on the mitochondria, this localization is not required for the regulation of apoptosis (Tan et al. 2007). In *D. melanogaster*, neither mitochondria nor Bcl-2 homologs regulate caspase activation. Instead, caspase activity is regulated primarily through interactions between caspases and inhibitor of apoptosis (IAP) proteins (Ryoo and Baehrecke 2010). Importantly, MOMP does not occur in *C. elegans* apoptotic cell death, and although MOMP has been observed during apoptosis in *D. melanogaster*, this is a consequence rather than a cause of caspase activation (Abdelwahid et al. 2007). This has led to the prevalent opinion that MOMP-dependent regulation of apoptosis evolved in higher eukaryotes. However, recent findings challenge this view; in the lophotrochozoan invertebrate *Planaria* (phylum Platyhelminthes), proapoptotic stimuli induce MOMP, and planarian caspases can be activated in cytosols by cytochrome *c* (unlike *D. melanogaster* or *C. elegans* caspases) (Bender et al. 2012). *Planaria* also encode a proapoptotic Bak homolog that can directly induce MOMP. Similarly, schistosomes (phylum Helminthes) also encode Bcl-2 proteins that can regulate MOMP (Lee et al. 2011). Cytochrome *c* can also activate caspases from an invertebrate deuterostome, the purple sea urchin, *Strongylocentrotus purpuratus* (phylum Echinodermata) (Bender et al. 2012). Collectively, these findings argue that, in cell death terms, *D. melanogaster* and *C. elegans* may be evolutionary outliers and that MOMP may be the primordial and predominant means of caspase activation in animals.

UNLEASHING THE DEATH SQUAD: MOLECULAR MECHANISMS OF MOMP

Because MOMP dictates cells fate, it is highly regulated, largely through interactions between pro- and antiapoptotic Bcl-2 family members (Youle and Strasser 2008). How antiapoptotic Bcl-2 proteins regulate MOMP is discussed elsewhere—here we review how the proteins that are required for MOMP, Bax and Bak, are activated and how, upon activation, they permeabilize the mitochondrial outer membrane.

Following activation by direct interaction with BH3-only Bcl-2 proteins, Bax and Bak undergo dramatic structural changes leading to mitochondrial targeting of Bax (which is predominantly cytosolic when inactive) and homo-oligomerization of Bax and Bak (Hsu et al. 1997; Eskes et al. 2000; Wei et al. 2000). Oligomerization of Bax and Bak is essential for MOMP because mutants that fail to oligomerize are completely inactive (George et al. 2007; Dewson et al. 2008). Given their pivotal role in deciding whether a cell dies or not, the mechanisms underlying Bax and Bak activation have been intensively investigated; however, it remains contentious how these proteins drive MOMP (Fig. 2). One model proposes that Bax is activated by BH3-only proteins, not by binding in the hydrophobic BH3-binding pocket of Bax (which might be expected) but rather by interacting on the opposite side of Bax (Gavathiotis et al. 2008, 2010). Activated Bax then self-propagates further activation through its own, newly exposed BH3-only domain. This leads to the formation of asymmetric Bax oligomers that ultimately cause MOMP. Alternatively, BH3 proteins can activate Bax and Bak by binding in their hydrophobic BH3-binding pockets (Czabotar et al. 2013; Leshchiner et al. 2013; Moldoveanu et al. 2013). Upon activation, Bax and Bak homodimerize in a head-to-head manner (Dewson et al. 2008, 2012). Dimerization unveils a cryptic dimer–dimer binding site that allows oligomers of homodimers to form and cause MOMP (Dewson et al. 2009).

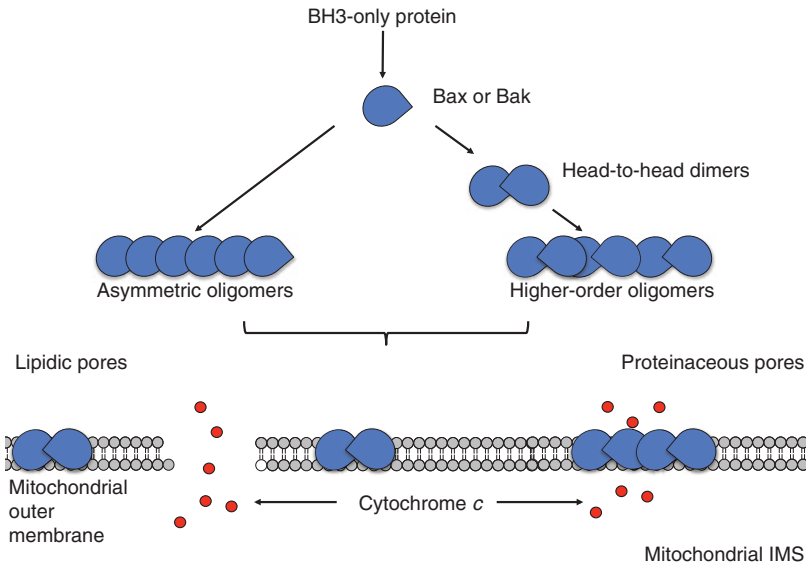

Figure 2. Mechanism of Bax/Bak activation and MOMP. BH3 domain-only proteins directly bind and activate Bax and Bak. Activated Bax and Bak form higher-order oligomers, either through asymmetric oligomers (Bax) or through the formation of higher-order oligomers formed by head-to-head Bax or Bak dimers. How oligomeric Bax and Bak permeabilize the mitochondrial outer membrane is unclear. Two prominent models argue that Bax and Bak do this either by inducing lipidic pores (*left*) or by directly forming proteinaceous pores (*right*).

Initial live-cell imaging studies, using cytochrome *c* GFP to report mitochondrial permeabilization, showed that, although the onset of MOMP is highly variable, following its initiation, permeabilization of mitochondria occurs in a rapid (<5 min) and complete manner (Goldstein et al. 2000). More recently, several studies have found that MOMP can occur at a defined point or points within a cell and propagate in a wave-like fashion over the whole cell (Lartigue et al. 2008; Bhola et al. 2009; Rehm et al. 2009). Exactly how these waves are propagated is unclear, but existing data argue against involvement of either caspases or the mitochondrial permeability transition, a change in the inner mitochondrial membrane permeability to small solutes (Crompton 1999). As discussed previously, the self-propagating nature of Bax and Bak activation might be expected to facilitate the occurrence of MOMP in a wave-like manner. Chemical inhibitors of casein kinase II inhibit wave formation, arguing that substrate(s) of this kinase (perhaps BH3-only proteins) are relevant for wave formation (Bhola et al. 2009). Alternatively, mitochondrial-derived reactive oxygen species (ROS) may promote wave formation because inhibition of ROS or addition of ROS scavengers prevents wave-like MOMP from occurring (Garcia-Perez et al. 2012). It remains unclear how permeabilization of individual mitochondria generates ROS, or, indeed, what the targets of ROS are that facilitate wave propagation.

Much interest has focused on whether MOMP permits selective or nonselective release of mitochondrial intermembrane space (IMS) proteins. At least in vitro, Bax-mediated permeabilization of liposomes leads to release of 10-kDa and 2-MDa dextrans with similar kinetics (Kuwana et al. 2002). In cells, proteins >100 kDa (predicted molecular weight of Smac-GFP dimers) are released with kinetics similar to cytochrome *c*; however, a Smac dsRed tetrameric fusion protein (predicted size 190 kDa) failed to be released from mitochondria upon MOMP (Rehm et al. 2003). Furthermore, ectopic expression of XIAP delays the kinetics of Smac release following MOMP from

mitochondria dependent on the ability of XIAP to enter the mitochondrial IMS and complex with Smac (Flanagan et al. 2010). Although these results suggest that the release of IMS proteins following MOMP may have size limitations in vivo, the onset of IMS protein release from mitochondria is the same irrespective of size, thus arguing that all soluble IMS proteins exit the mitochondria through a similar mechanism (Munoz-Pinedo et al. 2006). In some settings, selective release of mitochondrial IMS proteins can be observed; for example, cells deficient in Drp-1, a dynamin-like protein required for mitochondrial fission, preferentially release Smac but not cytochrome *c* following MOMP (Parone et al. 2006; Estaquier and Arnoult 2007; Ishihara et al. 2009). Why loss of Drp-1 selectively inhibits cytochrome *c* egress from the mitochondria remains unclear, but this can inhibit the kinetics of caspase activation and apoptosis. Interestingly, Drp-1 can also act as a positive regulator of Bax-mediated MOMP (Montessuit et al. 2010).

The requirement for Bax and Bak in MOMP is clear, but how these proteins actually permeabilize the mitochondrial outer membrane remains elusive. Two prominent models propose that activated Bax and Bak cause MOMP either by forming proteinaceous pores themselves or, alternatively, by causing the formation of lipidic pores in the mitochondrial outer membrane. As discussed above, pro- and antiapoptotic Bcl-2 proteins are structurally similar to bacterial pore-forming toxins, implying that Bax and Bak themselves might directly form pores in the mitochondrial outer membrane (Muchmore et al. 1996; Suzuki et al. 2000). Along these lines, several studies have found that Bax can induce ion channels in artificial membranes; however, somewhat confusingly, antiapoptotic Bcl-2 proteins can also form membrane pores (Antonsson et al. 1997). Patch-clamp studies of isolated mitochondria have discovered that during MOMP (initiated by the addition of the BH3-only protein tBid), a mitochondrial outer membrane channel forms that increases with size over time and displays kinetics similar to MOMP (Martinez-Caballero et al. 2009). This implies that the channel (termed the mitochondrial apoptosis-induced channel [MAC]) as the perpetrator of MOMP. In support of this, inhibitors that block MAC block MOMP and apoptosis in cells (Peixoto et al. 2009). However, it remains possible that these inhibitors block the initial activation of Bax and Bak. Furthermore, in the majority of studies, the size of the MAC channels detected have only been large enough to accommodate cytochrome *c* release, but, as discussed above, MOMP clearly allows for the release of much larger proteins.

An alternative model proposes that activated Bax and Bak cause MOMP by inducing lipidic pores. This model would account for various characteristics of MOMP including the release of large IMS proteins and a consistent inability to detect proteinaceous pores in the mitochondrial outer membrane. Activated Bax can induce liposome permeabilization in vitro, leading to the release of encapsulated material in a size-independent manner, thereby recapitulating a key characteristic of MOMP (Basanez et al. 1999, 2002; Hardwick and Polster 2002). Moreover, cryo-EM analysis of Bax-permeabilized liposomes revealed large openings (up to 100 nm). These appeared concurrently with permeabilization and could be inhibited in a Bcl-X_L-dependent manner (Schafer et al. 2009). In further support of the lipidic pore model, Bax-induced pores were variable in size and lacked proteinaceous material—this contrasts with protein pores formed by the bacterial toxin pneumolysin that are uniform in nature and proteinaceous in composition. However, whether activated Bax and Bak induce MOMP by forming lipid pores in mitochondrial outer membranes remains unclear because similar pore-like structures have not been observed in mitochondria.

APPETITE FOR DESTRUCTION: HOW MOMP KILLS CELLS

Irrespective of mechanism, MOMP wreaks havoc on the cell. Normally, MOMP leads to the release of proteins that activate caspases leading to rapid, apoptotic cell death. However, even in the absence of caspase activity, cells generally succumb to cell death through an ill-defined process termed caspase-independent cell death

(CICD) (Tait and Green 2008) (Fig. 1). Therefore, MOMP is often considered a point of no return. Here we review how MOMP triggers cell death through caspase-dependent and -independent means.

Mitochondrial-Dependent Caspase Activation

Although the onset of MOMP is highly variable, following mitochondrial permeabilization, caspases are activated in a robust manner leading to apoptosis typically within a few minutes (Goldstein et al. 2000; Albeck et al. 2008). Of the many mitochondrial intermembrane space proteins released following MOMP, cytochrome *c* is the most important. Once in the cytoplasm, cytochrome *c* transiently binds the key caspase adaptor molecule Apaf-1. This interaction triggers extensive conformational changes in Apaf-1 leading to its oligomerization into a heptameric wheel-like structure and exposure of caspase activation and recruitment domains (CARD) (Bratton and Salvesen 2010). The Apaf-1 CARD domains bind to CARD domains of the initiator caspase procaspase-9, forming the apoptosome. At the apoptosome, dimerization of caspase-9 leads to its activation, which, in turn, cleaves and activates the executioner caspases-3 and -7, leading to rapid cell death. Cytochrome *c* is essential for mitochondrial-dependent caspase activation; cells that lack cytochrome *c* or express a mutant that poorly activates Apaf-1 (but retains respiratory function) fail to activate caspases following MOMP (Li et al. 2000; Hao et al. 2005; Matapurkar and Lazebnik 2006). Moreover, mice expressing this mutated form of cytochrome *c* phenocopy the neurological defects observed in Apaf-1- and caspase-9-deficient mice.

Besides cytochrome *c*, other mitochondrial IMS proteins facilitate caspase activation. These include Smac (also called Diablo) and Omi (also called HtrA2) (Du et al. 2000; Verhagen et al. 2000; Suzuki et al. 2001). Both proteins reside in the mitochondrial intermembrane space and are released following MOMP. In healthy cells, Omi functions as a mitochondrial chaperone, whereas the nonapoptotic function for Smac is not known. Smac and Omi promote caspase activation by binding to and neutralizing the caspase inhibitor XIAP. However, in contrast to cytochrome *c*, loss of either Omi or Smac either individually or together does not impart resistance to caspase activation and apoptosis (Okada et al. 2002; Jones et al. 2003; Martins et al. 2004). Indeed, likely because of its chaperone function, cells and mice lacking Omi are rendered more sensitive to mitochondrial damage and cell death. Although these results argue that XIAP neutralization may facilitate rather than be essential for caspase activation, recent data argue that in death-receptor-triggered apoptosis, neutralization of XIAP is essential for effective caspase activation in type II cells (cells that require MOMP for death-receptor-induced apoptosis) (Jost et al. 2009). Moreover, there may be significant redundancy with respect to XIAP inhibition given the identification of various other mitochondrial proteins that can inhibit XIAP (Zhuang et al. 2013).

Other mitochondrial IMS proteins that have been proposed to facilitate caspase activation include apoptosis-inducing factor (AIF). In contrast to cytochrome *c*, the release of AIF from the mitochondrial IMS following MOMP is slow and, in some circumstances, caspase-dependent (Arnoult et al. 2003; Munoz-Pinedo et al. 2006). As such, AIF likely does not seem to play a major role in apoptosis induction.

Even in the absence of caspase activity, cells typically succumb to a slower, ill-defined form of death termed caspase-independent cell death (CICD). CICD may serve primarily as a fail-safe mechanism to ensure that cell death occurs even if caspases are inhibited (e.g., by a viral caspase inhibitor). Careful morphological analysis revealed that under physiological conditions, CICD may account for up to 10% of cell death—if this is, indeed, the case, it represents a major cell death modality (Chautan et al. 1999). Furthermore, comparison of early embryonic lethality (typically embryonic day 7 [E7], although some survive and can mature to adulthood) observed with Bax/Bak-deficient mice (unable to undergo MOMP) with the postnatal lethality of Apaf-1-deficient mice (can only undergo CICD) argues that, at the gross level,

CICD can effectively substitute for apoptosis, at least during development (Yoshida et al. 1998; Lindsten et al. 2000). That said, the ~15% of Bax/Bak-deficient animals that survive embryogenesis and mature, showing some neurological defects and expansion of lymphoid cells, represents an ongoing puzzle for the role of MOMP in development.

How CICD occurs following MOMP is unclear. Indeed, the mechanism of CICD may vary in a cell-type-dependent manner—unlike the canonical, mitochondrial pathway of caspase activity. One model supports an active role for mitochondria in mediating cell death, for example, through the release of proteins following MOMP such as AIF that can actively induce CICD. AIF may contribute to caspase-independent cell death (CICD) in some settings (Cheung et al. 2006). Alternatively, CICD may be mediated primarily by mitochondrial dysfunction that ensues following MOMP, ultimately leading to metabolic catastrophe and cell death. Along these lines, analysis of cells undergoing CICD found a rapid reduction in mitochondrial respiratory complex I and IV function (Lartigue et al. 2009). At subsequent time points post-MOMP, cytochrome *c* can be targeted for proteasome-dependent degradation, again promoting respiratory dysfunction (Ferraro et al. 2008). In addition to breakdown of mitochondrial respiratory function, mitochondrial proteins including TIM23 (an essential component of the mitochondrial inner membrane translocase complex) can be cleaved and inactivated following MOMP, in doing so contributing to mitochondrial dysfunction (Goemans et al. 2008). Moreover, given the important role that AIF has in maintaining respiratory complex I function (Vahsen et al. 2004), loss of AIF from the mitochondria should also promote mitochondrial dysfunction. Collectively, these findings argue that loss of mitochondrial function may be the principle reason that cells die through CICD following MOMP. However, because cells can survive complete removal of mitochondria for at least 4 d, which is typically longer than the kinetics of CICD, this still suggests that permeabilized mitochondria may also play an active role in CICD (Narendra et al. 2008). One such role may be as "ATP-sinks" because maintenance of the transmembrane potential is sustained by reversal of the F_0F_1 ATPase.

POST-MOMP REGULATION OF CASPASE ACTIVITY

Under some circumstances, MOMP need not be a death sentence. However, in order to evade cell death post-MOMP, cells must limit caspase activation. Here we review mechanisms of caspase activity regulation after MOMP, focusing on regulation of IMS protein release following MOMP and direct means of inhibiting caspase activation following mitochondrial permeabilization.

Post-MOMP Regulation of IMS Protein Release

MOMP itself does not appear to afford any specificity over which IMS proteins are released from the mitochondria. However, various studies implicate mechanisms that govern selective release of IMS proteins following MOMP; principally, these mechanisms center on IMS protein interaction with the mitochondrial membranes or by remodeling of the mitochondrial inner membrane (Fig. 3).

AIF is tethered to the mitochondrial inner membrane; consequently, its release following MOMP requires proteolytic cleavage either by caspase or calpain proteases (Arnoult et al. 2003; Polster et al. 2005). In the case of cytochrome *c*, electrostatic interactions with inner membrane lipids and the oxidative state of these lipids (where oxidized lipids bind cytochrome *c* less) have been proposed to regulate its release following MOMP (Ott et al. 2002).

The mitochondrial inner membrane is largely composed of cristae, involutions that greatly expand the mitochondrial surface area for oxidative phosphorylation and ATP generation. Far from being static, cristae are highly dynamic structures, and their accessibility to the IMS is regulated through cristae junctions. Interestingly, most cytochrome *c* resides in mitochondrial cristae, leading various studies to

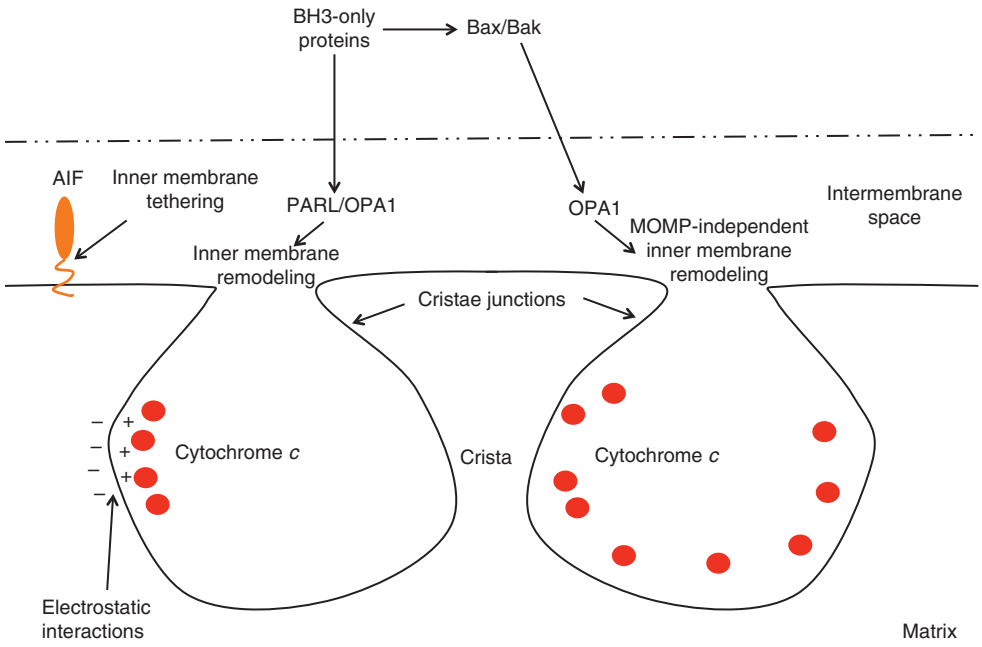

Figure 3. Post-MOMP regulation of mitochondrial intermembrane space protein release. The intermembrane space protein AIF is tethered to the mitochondrial inner membrane and requires cleavage to liberate it from the mitochondria upon MOMP. The majority of cytochrome *c* is sequestered within mitochondrial cristae; electrostatic interactions facilitate its association with the inner membrane. Some studies argue that cristae remodeling must occur to allow cytochrome *c* egress from the mitochondrial cristae following MOMP. Cristae remodeling can occur in a MOMP-independent manner by BH3 proteins (in a Bax/Bak-independent manner) or by activated Bax and Bak. Remodeling is dependent upon the intermembrane space rhomboid protease PARL and the dynamin-like GTPase OPA1.

address whether cristae remodeling provides an additional layer of regulating cytochrome *c* release from the mitochondria. Accordingly, several BH3-only proteins including Bid, Bim, BNIP3, and Bik have been found to regulate cristae remodeling (Scorrano et al. 2002; Germain et al. 2005; Yamaguchi et al. 2008). In vitro treatment of mitochondria with the BH3 protein tBid leads to extensive remodeling, interconnected cristae, and cytochrome *c* mobilization from the cristae into the IMS. Interestingly, this effect of tBid on mitochondrial inner membrane dynamics did not require the tBid BH3 domain (Scorrano et al. 2002). Other studies have found that membrane remodeling requires active Bax and Bak but does not necessitate MOMP, because pharmacological inhibitors of MOMP still allow remodeling (Yamaguchi et al. 2008). Two IMS proteins, OPA1 (a dynamin-like GTPase) and PARL (a rhomboid protease), are essential for regulating cristae dynamics. Upon MOMP, disruption of OPA1 oligomers widens cristae junctions, whereas PARL cleavage of OPA1 generates a cleavage product that maintains tight junctions (Frezza et al. 2006). However, other studies have found no gross changes in mitochondrial morphology or cristae junction size upon MOMP or only detected them following executioner caspase activity—this argues that remodeling may be consequential rather than causative in promoting IMS protein release (Sun et al. 2007). Moreover, even in a closed state, cytochrome *c* should be able to exit cristae junctions, arguing that cristae width is not a key determinant of release in itself (Gillick and Crompton 2008). Possibly, cristae remodeling may support IMS protein release in a cell-type-specific manner, or OPA1 and PARL

may facilitate IMS protein release independently of cristae remodeling.

Besides regulating IMS protein release post-MOMP, a plethora of mechanisms have been described that can limit caspase activity. The physiological role of these mechanisms is uncertain, but perhaps they serve to restrain caspase activity and allow viability should MOMP occur in a limited number of mitochondria. As discussed above, through a well-described mechanism, XIAP can limit caspase activation by binding active caspases-9, -3, and -7. However, additional direct and indirect means of regulating caspase activity also exist that center on the formation and activation of the apoptosome. Importantly, various means of inhibiting apoptosome activation have been described in cancer, implying that this may facilitate cancer cell survival (Schafer and Kornbluth 2006).

Apoptosome Formation: Regulating the Wheel of Misfortune

Formation of the apoptosome is essential for efficient caspase-9 activation and mitochondrial-dependent apoptosis. APAF1 must bind dATP for apoptosome formation; however, paradoxically, physiological levels of nucleotides inhibit apoptosis by directly binding cytochrome *c*, preventing it from binding APAF1 (Chandra et al. 2006) (Fig. 4). Similarly, transfer RNA (tRNA) has also been found to bind cytochrome *c*, blocking its interaction with APAF1 and thereby preventing apoptosome formation (Mei et al. 2010). Physiological levels of potassium and calcium also inhibit cytochrome *c*-induced apoptosome formation (Cain et al. 2001; Bao et al. 2007). These inhibitory mechanisms may primarily exist to suppress accidental MOMP-induced caspase activity but are overwhelmed following rapid and extensive mitochondrial release of cytochrome *c* during apoptosis.

The redox status of a cell may also affect the proapoptotic activity of cytochrome *c* where oxidation promotes its proapoptotic activity and reduction inhibits it (Pan et al. 1999; Borutaite and Brown 2007). Mechanistically, how redox status would affect the ability of cytochrome *c* to induce apoptosome formation remains unclear, and some studies have found that reduced cytochrome *c* can still effectively activate caspases in vitro (Kluck et al. 1997). Various other proteins including HSP70, HSP90, and Cdc6 have been found to inhibit apoptosome function either by blocking its assembly or by inhibiting binding and activation of procaspase-9 at the apoptosome (Beere et al. 2000; Pandey et al. 2000; Saleh et al. 2000; Niimi et al. 2012).

Apoptosome function can also be positively regulated. The protein PHAP1 (also known as pp32) enhances apoptosome function by inhibiting aggregation of APAF1 and promoting nucleotide exchange (Jiang et al 2003; Kim et al. 2008). Importantly, reduced levels of PHAP1 inhibit apoptosis and allow clonogenic survival following chemotherapy—this finding may be relevant in small cell lung cancer because reduced PHAP expression correlates with poor clinical response to chemotherapy (Hoffarth et al. 2008).

Regulating Caspase-9 Activation

In addition to regulation of apoptosome assembly, caspase-9 activity can also be regulated. Several kinases can phosphorylate caspase-9 and inhibit its enzymatic activity. These include the MAP kinases ERK1 and ERK2 and CDK1-cyclin B1 (Allan et al. 2003; Allan and Clarke 2007). Although it is clear that phosphorylation can inhibit caspase-9 activity, how it achieves this is not understood. Because recruitment of procaspase-9 to the apoptosome does not appear to be affected by phosphorylation, perhaps phosphorylation of caspase-9 blocks its ability to dimerize. Interestingly, Rsk kinase (also a member of the MAPK family) has been found to inhibit Apaf-1 function by direct phosphorylation (Kim et al. 2012). This enables the adaptor protein 14-3-3ε; to bind Apaf-1 and prevent apoptosome assembly. At the apoptosome, autoprocessing of caspase-9 leads to a dramatic reduction in its affinity for the apoptosome, resulting in loss of caspase-9 activity. This mechanism acts as a "molecular timer" of which its activity (and ability to drive executioner caspase activity) is dictated by intracellular caspase-9

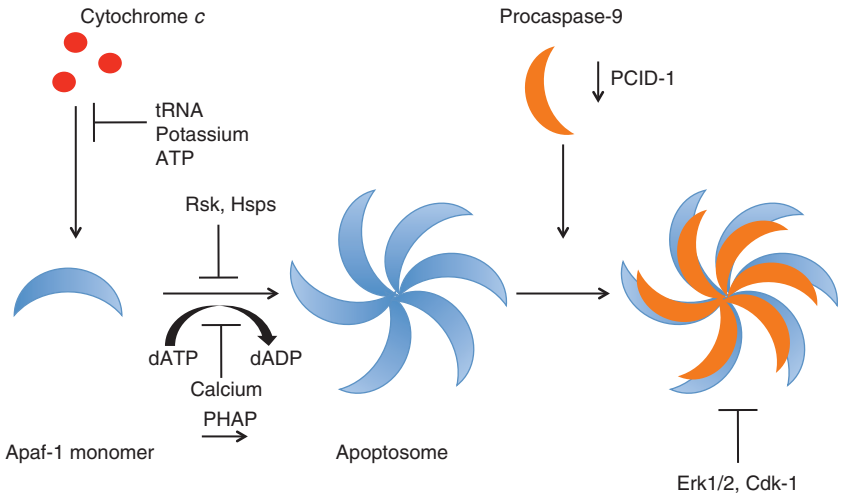

Figure 4. Regulation of apoptosome activity. Various molecules, including tRNA, potassium, and ATP, can competitively inhibit cytochrome *c*–Apaf-1 interactions, thereby blocking apoptosome formation. Apaf-1 oligomerization can be positively affected by proteins such as PHAP that facilitate nucleotide exchange, whereas intracellular calcium levels inhibit this event. Various proteins, including heat shock proteins (Hsps) and kinases such as Rsk can directly inhibit Apaf-1 oligomerization through interaction with Apaf-1 or by inhibitory phosphorylation. The activity of the apoptosome can also be inhibited by the kinase activity of Erk1/2 and Cdk-1. Finally, proteins such as PCID1 can regulate the intracellular levels of procaspase-9, thereby regulating apoptosome activity.

levels (Malladi et al. 2009). Consequently, regulation of caspase-9 expression can also control caspase activity post-MOMP. PCID1 is the human ortholog of Tango7, a *D. melanogaster* protein that regulates expression of the initiator caspase pro-Dronc (Chew et al. 2009). In an analogous manner, down-regulation of PCID1 reduces expression of procaspase-9. This may be clinically relevant because PCID1 is frequently down-regulated in pancreatic cancer (Jones et al. 2008).

DODGING THE BULLET—CELL SURVIVAL FOLLOWING MOMP

Although MOMP often represents a point of no return, this is not always the case. Cell survival following MOMP likely has important pathophysiological functions by facilitating long-term survival of postmitotic cells and enabling tumor cell survival. Moreover, MOMP itself may have noncytotoxic signaling functions, thereby requiring cells to survive this process. Here we discuss how cells survive MOMP and the roles, both good and bad, that survival post-MOMP can have.

Surviving "Accidental" MOMP

Live-cell imaging studies led to the initial view that MOMP is an all-or-nothing event (Goldstein et al. 2000). However, subsequent work has found that MOMP can sometimes be incomplete, leaving a minority of mitochondria intact (Tait et al. 2010). This suggests that the converse could also occur; limited mitochondria may undergo permeabilization without leading to cell death. Such accidental MOMP would necessitate that a threshold extent of MOMP must be crossed in order to trigger apoptotic caspase activity. Indeed, laser irradiation of neuronal mitochondria leading to MOMP of 15% of a cell's mitochondria was insufficient to trigger MOMP (Khodjakov et al. 2004). As already discussed, there are a plethora of mechanisms that can restrain caspase activity post-MOMP, but whether MOMP does occur in a few mitochondria without triggering cell death remains unknown.

Postmitotic Cell Survival

The life-long requirement of postmitotic cells necessitates robust prosurvival mechanisms. Both sympathetic neurons and cardiomyocytes can survive MOMP, at least in part, because they express insufficient levels of APAF-1 to activate caspases efficiently (Wright et al. 2004; Potts et al. 2005). XIAP is also a major player in conferring nonresponsiveness to MOMP in these cell types because addition of SMAC or deletion of XIAP can restore apoptotic sensitivity (Potts et al. 2003). In the case of neurons, NGF deprivation induces a so-called competence to die because it leads to XIAP down-regulation (Deshmukh and Johnson 1998; Martinou et al. 1999). Besides XIAP, the high glycolytic levels of neurons also facilitate inhibition of caspase activity (Vaughn and Deshmukh 2008). Glycolysis leads to increased glutathione synthase levels through the pentose phosphate shunt. As discussed above, reduction of cytochrome *c* can impair its ability to induce apoptosome activation. Comparable inhibitory mechanisms may also play a role in tumor cells given that they too are highly glycolytic.

Recovery from MOMP in Dividing Cells

In some situations, proliferating cells can survive MOMP provided that caspase function is inhibited. This has the potential to have an impact on both tumor development and therapeutic responses because cancer cells often inhibit caspase activity downstream from MOMP by a variety of mechanisms. Through a retroviral-based cDNA screen, GAPDH was found to protect cells from caspase-independent cell death downstream from MOMP (Colell et al. 2007). This protective role of GAPDH was due both to its well-established role as a key glycolytic enzyme and a newly described function by up-regulating autophagy. The ability of GAPDH to promote cell survival may be important in BCR-ABL-dependent chronic myeloid leukemia because GAPDH can promote resistance to cell death induced by BCR-ABL inhibitors (Lavallard et al. 2009).

Numerous events must occur in order for a cell to survive MOMP. Permeabilized mitochondria must be repaired or removed, and "new" mitochondria must be generated. Mitochondrial repopulation requires a cohort of mitochondria that fail to permeabilize following MOMP. The ability of certain mitochondria to evade MOMP relates to increased levels of antiapoptotic Bcl-2 proteins on their outer membrane; accordingly, Bcl-2 antagonist drugs can effectively permeabilize these mitochondria. Together with the strong correlation observed between the presence of intact mitochondria and cell survival, this suggests that the intact mitochondria provide a seed population of healthy mitochondria that ultimately repopulate the cell (Tait et al. 2010).

SUMMARY

Our understanding of MOMP and how it triggers cell death has advanced to the stage that drugs have now been developed to target this process. Nevertheless, significant gaps in our knowledge exist. For example, how activated Bax and Bak permeabilize the mitochondrial outer membrane is unknown. Secondly, although we understand how MOMP drives caspase activation, we have little mechanistic insight as to how it leads to CICD. The extent to which cells undergo CICD in vivo is difficult to gauge, mainly because of the lack of tools to detect and quantify this form of cell death accurately. Furthermore, although poorly understood, much greater attention is now being paid to how the mode of cell death influences the way the immune system perceives and reacts to a dying cell. Last, as we have discussed, MOMP need not be a death sentence. However, the mechanisms that allow cells to recover from MOMP remain poorly defined, as do its in vivo occurrence and pathophysiological importance. Ultimately, further understanding of how MOMP dictates life and death will facilitate its therapeutic targeting in a variety of diseases.

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