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Greasing the Path to BAX/BAK Activation

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BAX/BAK activation leading to mitochondrial outer-membrane permeabilization is a key commitment point in apoptosis. Chipuk et al. now identify two sphingolipids as specific cofactors for BAX/BAK activation that lower the threshold for apoptosis-associated cytochrome *c* release. Association of mitochondria with other cellular membrane compartments is required for BAK/BAX exposure to these sphingolipids.

BAX/BAK activation is a major checkpoint in apoptosis, as this event irreversibly commits the majority of cells to die (Llambi et al., 2011). Upon activation, BAX and BAK oligomerize within the mitochondrial outer membrane, resulting in the formation of a pore or channel that promotes release of cytochrome *c* and many other proteins from the mitochondrial intermembrane space. This process is known as mitochondrial outer-membrane permeabilization (MOMP). In the cytosol, cytochrome *c* acts as a cofactor for the assembly of a caspase-activating complex called the apoptosome, which triggers a proteolytic cascade that coordinates cell destruction typical of apoptosis. In addition, MOMP causes mitochondria to rapidly depolarize and lose their capacity to generate ATP, which also proves fatal for the majority of cells, irrespective of whether the apoptosome is activated downstream. Much effort has therefore been directed toward understanding BAX/BAK oligomerization and the factors that promote or oppose BAX/BAK-dependent MOMP. In this issue of *Cell*, Chipuk et al. report that the sphingolipid metabolites, sphingosine-1-phosphate and hexadecenal, act as cofactors for BAK and BAX activation, respectively (Chipuk et al., 2012).

BAX/BAK oligomerization within the mitochondrial outer membrane is typically initiated due to stress or damage that activates one or more members of the BH3-only subset of the Bcl-2 family (Youle and Strasser, 2008). BH3-only proteins serve as pathway-specific stress sensors that promote activation of BAX and/or BAK either through direct interaction with the latter proteins or through binding and neutralizing BAX/BAK antagonists such as Bcl-2 and its close relatives (Letai et al., 2002; Llambi et al., 2011). Recent studies suggest that extensive contacts exist between mitochondria and other membrane compartments, such as the ER, which facilitates the transfer of calcium as well as lipids between these organelles and can influence signal propagation within the cell (de Brito and Scorrano, 2008). To explore the factors that influence the threshold for MOMP, Chipuk et al. compared the efficiency of BH3-only protein-induced BAK activation in mitochondrial preparations containing diverse membranes, including those from the ER and other membrane compartments, with highly purified mitochondria free of heterotypic membranes (Chipuk et al., 2012). BAK activation was greatly compromised in the highly pure mitochondrial preparations when compared with mitochondria containing ER and other microsomal membranes.

To parse out which components of the heterotypic membranes facilitated BAK activation, the authors carried out fractionations and identified two neutral sphingomyelinases (SMases) enriched in a subfraction capable of restoring sensitivity to MOMP in purified mitochondria (Chipuk et al., 2012). Moreover, addition of purified neutral SMase was sufficient to restore sensitivity to BH3-only protein-induced BAK activation. SMases hydrolyze sphingomyelin to ceramide, a lipid that has previously been implicated as a second messenger in apoptosis, although the mechanistic basis for the proapoptotic effects of ceramide has been debated (Taha et al., 2006). Ceramide can undergo further conversion to sphingosine and sphingosine-1-phosphate (S1P) through the actions of ceramidase and sphingosine kinase, respectively. Using a panel of enzymes and inhibitors to walk down the sphingomyelin hydrolysis pathway, Chipuk et al. identified S1P and its breakdown product, hexadecenal (Hex), as factors that can promote BAK and BAX oligomerization, respectively. Furthermore, manipulation of sphingolipid metabolism in cells either by overexpression of neutral SMases or by knocking down a mitochondrial-associated sphingosine kinase (SPHK2) required for S1P production led to sensitization or protection from apoptosis, respectively (Chipuk et al., 2012).

Collectively, these observations suggest that mitochondria-associated organelles, such as the ER, can influence the set point for apoptosis through tonic provision of sphingolipids to mitochondria that influence BAX/BAK oligomerization thresholds (Figure 1). Although the SMase activity required for ceramide generation appears to reside in a mitochondrial-associated membrane compartment, further conversion of ceramide through to S1P and Hex is likely to occur within mitochondrial membranes (Chipuk et al., 2012). Although sphingomyelin pathway metabolites have been implicated in apoptosis in many previous studies (Taha et al., 2006), a consensus view on how these lipids influence apoptosis has

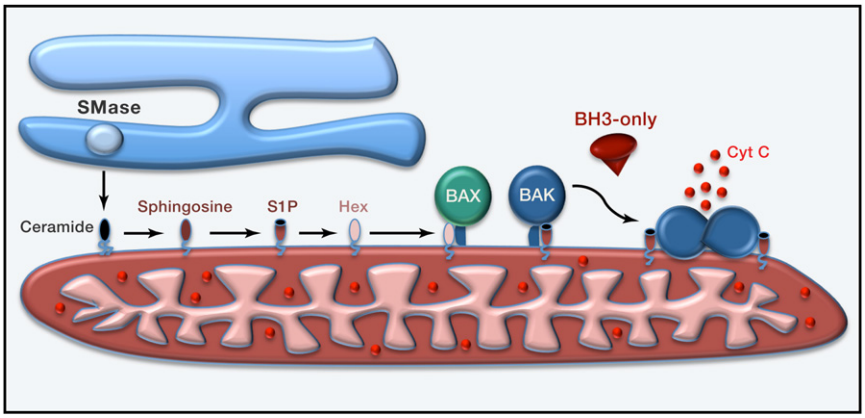

Figure 1. Sphingolipids Lower the Threshold for BAX and BAK Activation
Mitochondrial-associated membrane compartments contain sphingomyelinases (SMases) that provide sphingolipids to mitochondria. SMases convert sphingomyelin to ceramide, which can be further converted to sphingosine via ceramidase activity. Sphingosine kinase activity converts sphingosine to S1P, which can be converted to hexadecenal (Hex) via S1P lyase. Note that reverse flux from S1P to sphingomyelin is also possible. Hex binds specifically to BAX and promotes BAX oligomerization upon exposure to BH3-only proteins, whereas S1P promotes BAK oligomerization upon BH3-only exposure. Its specific binding to BAK has not yet been explored. Oligomerization of either BAK or BAX results in cytochrome *c* (Cyt *c*) release; only a BAK complex is shown here for simplicity.

been lacking. The present study suggests that sphingolipids function to provide the correct milieu within mitochondrial outer membranes that permits efficient BH3-only protein-initiated BAX/BAK oligomerization (Figure 1). Thus, forward or reverse flux through the sphingolipid pathway may increase or decrease the threshold for apoptosis by altering the levels of S1P and Hex that are available for BAK and BAX binding.

But specifically, how do Hex and S1P lower the threshold for BAX/BAK activation? Oligomerization of BAX and BAK is associated with major conformational changes within their N termini that expose BH3 domains and permit interaction with the surface hydrophobic grooves of a neighboring BAX or BAK molecule (reviewed in Dewson and Kluck, 2009). Additional "back-to-back" interactions between BAX or BAK dimers promote further oligomerization to form pores that permit efflux of mitochondrial constituents (Dewson and Kluck, 2009). Although Chipuk et al. did not explore this issue beyond demonstrating that Hex can selectively bind to BAX and facilitate a conformational change typically observed at the onset of MOMP, it seems reasonable to propose that BAX and BAK contain lipid-binding pockets that, when occupied, lower the energetic constraints required to undergo these transitions. Alternatively, S1P- and Hex-enriched microdomains may exist on mitochondrial outer membranes that permit more efficient assembly of BAX or BAK oligomers within these regions.

With regard to the possibility of direct binding of S1P or Hex to BAK and BAX, it is noteworthy that a recent study identified a recognition sequence for sphingomyelin 18 within the transmembrane domain of the COPI machinery protein p24, which resides within the membrane of Golgi-derived vesicles (Contreras et al., 2012). Sphingomyelin binding to p24 regulated its propensity to switch from a monomeric (inactive) to an oligomeric (active) state (Contreras et al., 2012). Thus, in addition to fulfilling structural roles within cell membranes, sphingolipids may serve as exquisitely specific cofactors for membrane proteins that regulate the activation state of such molecules. In future studies, it will be interesting to explore whether BAX or BAK have similar sphingolipid-binding domains.

Interestingly, studies have also suggested that S1P production is increased during apoptosis and, upon secretion, helps to guide phagocytes toward dying cells to facilitate their swift removal from tissues (Weigert et al., 2010; Gude et al., 2008). S1P accumulation during apoptosis has been reported to be due to upregulation of the sphingosine kinase SPHK1, as well as caspase-dependent proteolysis of SPHK2 (Gude et al., 2008; Weigert et al., 2010). It is tempting to speculate that SPHK activation, leading to S1P generation, may kill two birds with one stone during apoptosis. On the one hand, increased S1P production may lower the threshold for BAK activation, and on the other, S1P secretion may help to coordinate the safe disposal of the dying cell through acting as a "find me" signal for phagocytes.

Understanding the mechanics of BAX/BAK activation has proved challenging, and important questions clearly remain. The precise composition and structure of the fully assembled BAX/BAK mitochondrial channel await definition. It is also unclear whether this channel is comprised solely of BAX and/or BAK or contains additional constituents. However, the discovery that BAX and BAK activation is regulated by sphingolipids may go some way toward explaining why unraveling the intricacies of MOMP has proven to be quite a slippery task.

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