The peroxisomes strike BAK: Regulation of peroxisome integrity by the Bcl-2 family

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Within the mitochondrial pathway of apoptosis, VDAC2 controls both the localization and proapoptotic activity of BAK. In this issue, Hosoi et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201605002) find that loss of VDAC2 diverts BAK into peroxisome membranes, revealing the ability of BAK to control peroxisome membrane integrity and the release of soluble peroxisomal matrix proteins.

Thousands of proteins, hundreds of posttranslational modifications, different dynamic membranes, and myriad environmental cues orchestrate organelle biology. Despite this diversity, conserved proteins and pathways weave consistency through the ostensibly chaotic nature of the cytoplasm. For example, similar GTPases control the distinct dynamic processes of fusion and fission that shape the mitochondrial and peroxisomal networks. Not to mention the complex and overlapping pathways of mitophagy and pexophagy that are responsible for the removal of marred mitochondria and poorly peroxisomes. Yet, we are always surprised when additional shared functions of a protein or pathway are revealed between organelles. In this issue, Hosoi et al. discover that B cell lymphoma 2 (BCL-2) proteins, regulators of the mitochondrial pathway of apoptosis, also regulate peroxisomal membrane permeability and efflux of peroxisomal matrix proteins into the cytosol.

Peroxisomes are membrane-bound organelles containing a multitude of enzymes, including those contributing to fatty acid β-oxidation, lipid biosynthesis, and the conversion of hydrogen peroxide to water and oxygen via catalase (Smith and Aitchison, 2013). These organelles are thought to assemble in a manner similar to mitochondria, meaning they import and sort proteins into their membrane and matrix compartments by the use of peroxisome-specific targeting signals, receptors, and translocation machinery. The importance of robust peroxisome assembly for organismal health is highlighted by a cohort of peroxisome biogenesis disorders (e.g., Zellweger Syndrome) that affect a host of tissues and organs in development; at present these diseases are not curable (Waterham et al., 2016).

Regulation of membrane permeabilization was the original function identified for the BCL-2 family, but this was thought to be unique to the outer mitochondrial membrane (OMM; Luna-Vargas and Chipuk, 2016). The BCL-2 family is composed of ∼20 proteins that are divided into antiapoptotic (e.g., BCL-xL) and proapoptotic (e.g., BAK, BAX, and BIM) members. If the antiapoptotic proteins are neutralized by a subset of inhibitory proteins or small molecules, the proapoptotic BCL-2 proteins collaborate to create proteolipid pores in the OMM. For example, BAK usually resides in the OMM, and upon interaction with a stress-induced BH3 (BCL-2 homology domain 3)-only protein (e.g., BIM) and distinct lipids within the OMM, it undergoes conformational changes leading to homo-oligomerization and pore formation. Soluble proteins within the mitochondrial inner membrane space, such as cytochrome *c*, are then permitted access to the cytosol, leading to the activation of caspases and subsequent commitment to apoptosis. Antiapoptotic proteins, for example, BCL-xL, prevent the mitochondrial pathway of apoptosis by binding BAK and/or BIM, therefore stopping pore formation in the OMM.

For nearly fifteen years, voltage-dependent anion channel 2 (VDCA2) has been intimately linked to the function of BAK (Cheng et al., 2003; Naghdi et al., 2015). Most literature focuses on the role of VDAC2 as an essential channel for metabolite and ion flux across the OMM. Interestingly, the genetic deletion of *Vdac2* (and not *Vdac1* or *Vdac3*) causes embryonic lethality, arguably through the combined disruption of ion transport and apoptosis. VDAC2 likely controls the mitochondrial localization and function of BAK to govern the mitochondrial pathway of apoptosis. But what is the fate of BAK and its function when VDAC2 is removed from mitochondria?

Tateishi et al. (1997) previously generated a variant of the CHO cell line named ZP114 that displayed impaired import of peroxisome matrix proteins. Hosoi et al. (2017) screened a human kidney cDNA library in ZP114 cells to identify a gene that restored peroxisomal function. They discovered that VDAC deficiency was the reason for peroxisome dysfunction, and reconstitution with *Vdac2* cDNA was sufficient to correct the peroxisome phenotype. The requirement for VDAC2 in promoting peroxisome integrity was also confirmed in rat cells and in *Vdac2*$^{-/-}$ mouse embryonic fibroblasts (MEFs). Although the peroxisome phenotype in *Vdac2*$^{-/-}$ MEFs was observed in only a subset of cells (∼60%), the severity of peroxisomal dysfunction was stronger in MEFs compared with ZP114 cells because of additional problems in membrane protein targeting and sorting. Overall, these observations suggest that VDAC2 regulates several aspects of peroxisome biogenesis, with degrees of severity potentially linked to species and/or tissue of origin.

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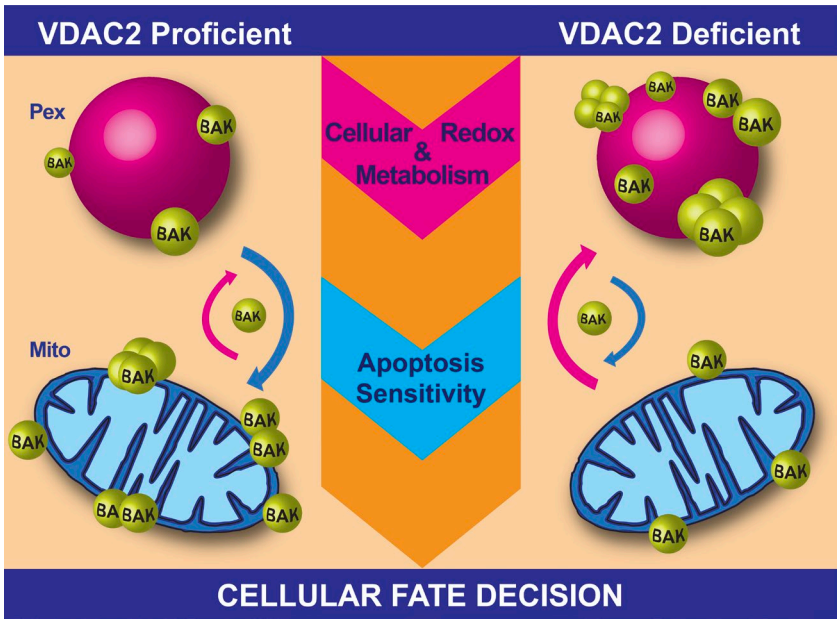

Figure 1. **VDAC2 controls BAK localization and function to differentially modulate peroxisomal (Pex) and mitochondrial (Mito) permeabilization.** When conditions allow for VDAC2 expression (left), BAK is primarily localized to the mitochondrial network to maintain appropriate sensitivity to apoptosis. Yet, a subpopulation of BAK also constitutively localizes to the peroxisomal network to maintain basal cytosolic catalase levels and cellular redox. In contrast, VDAC deficiency favors the accumulation of BAK at the peroxisomal network, which likely supports increased mitigation of redox and alterations in peroxisome-regulated cellular metabolism (right). Together, the VDAC2–BAK axis likely integrates multiple pathways to control cellular fate decisions.

The mechanistic link between peroxisome integrity and VDAC2 was not obvious from the initial experiments, as VDAC2 was not detected at peroxisomal membranes using organelle fractionation and microscopy-based techniques. This suggested that VDAC2 in mitochondria may regulate peroxisome integrity indirectly. Given the literature on VDAC2-dependent BAK targeting to mitochondria (Roy et al., 2009; Naghdi et al., 2015), Hosoi et al. (2017) hypothesized that the absence of VDAC2 in ZP114 cells could relocalize BAK to peroxisomes. Indeed, the silencing of *Bak* restored peroxisome integrity and corrected multiple aspects of peroxisome dysfunction. Transient expression of antiapoptotic BCL-2 proteins (e.g., BCL-xL and MCL-1) also restored the peroxisome defects in ZP114 cells, presumably through binding and inhibiting BAK either in the cytosol or within the peroxisomal membrane. Further investigations revealed BAK accumulation in both the cytosol and peroxisome network, and this was not altered upon coexpression with BCL-xL, suggesting that antiapoptotic BCL-2 proteins do not function akin to VDAC2 to alter BAK localization, but rather BCL-xL/MCL-1 inhibit aberrantly localized BAK in situ.

Several gain-of-function approaches were used in wild-type CHO and HeLa cells to examine the effects of BAK relocalization in cells that have a functional peroxisomal network. A fusion of BAK with Pex26p (a peroxisomal membrane protein involved in matrix protein import) was sufficient to localize BAK to the peroxisomal network. Expression of BAK-P26 disrupted peroxisomal integrity in ZP114 cells and caused the release of peroxisomal matrix proteins into the cytosol, such as catalase. This activity required the BH3 domain of BAK, which is a well-documented proapoptotic α-helical motif in BAK necessary for BAK homooligomerization, pore formation, and commitment to the mitochondrial pathway of apoptosis. The requirement for the BAK BH3 domain also corroborates the observation that antiapoptotic BCL-2 proteins block peroxisomal disruption by BAK as BCL-xL/MCL-1 directly sequester this domain.

A key next step after making an interesting observation in cell biology is to determine the physiological impact of the process in a normal setting. Hosoi et al. (2017) observed that BAK could localize to peroxisomes and regulate their integrity in wild-type CHO cells. *Bak* expression was silenced by RNA interference in HeLa cells, and the results suggest that endogenous BAK can balance the levels of cytosolic and peroxisome-localized catalase. As endogenous BAK is localized to the peroxisomal network, this raises the question: Can these BAK molecules respond to stress-induced BH3-only proteins? Hosoi et al. (2017) addressed this question by overexpressing multiple BH3-only proteins (e.g., BIM), which resulted in the redistribution of catalase from the peroxisomal matrix to the cytosol. These results suggest that peroxisome-localized BAK is fully competent to oligomerize and induce peroxisomal membrane permeability in a manner similar to mitochondrial BAK. However, pore formation by BAK at the peroxisome has yet to be demonstrated directly. Although multiple BCL-2 family subtypes (e.g., BH3-only proteins and antiapoptotic BCL-2 proteins) cooperate to influence the integrity of the peroxisome network, it should be noted that removal of BAX does not rescue the peroxisome deficiency in *Vdac2*-deficient cells, emphasizing that this is a unique function for BAK.

Although we have some indication as to which BCL-2 proteins control the permeabilization of peroxisomes, there is little known about the impact this has on cellular signaling and function. There are at least three scenarios worthy of discussion. First, what is the fundamental contribution of peroxisome-localized BAK to the biology of the cell in the absence of cellular stress? Hosoi et al. (2017) suggest basal levels of BAK activation promote the constitutive release of low levels of catalase into the cytosol. This is an attractive model, as mammalian cells lack a cytosolic catalase, which is present in lower organisms like yeast and worms (Hartman et al., 2003). Perhaps BAK-dependent release of catalase reduces cytosolic hydrogen peroxide to balance cellular redox signaling. Second, once stress-induced BH3-only proteins are expressed and/or activated, are there scenarios where BAK is not competent to promote the mitochondrial pathway of apoptosis and perhaps contributes to overall survival by mitigating either enhanced cellular redox or altering other functions of the peroxisome network that may reduce stress or encourage cellular repair mechanisms? Third, is it plausible that physiological or pharmacological inhibitors of the BCL-2 family or VDAC2 (e.g., Navitoclax and Erastin, respectively) alter cellular

redox and metabolism to influence human disease and/or therapy through this peroxisomal function of BAK, rather than via mitochondria (Yagoda et al., 2007; Luna-Vargas and Chipuk, 2016)?

In summary, must we unlearn what we know about proapoptotic members of the BCL-2 family? It is likely that we need to reevaluate the definitions of some proapoptotic BCL-2 proteins and their regulatory binding partners (i.e., the BAK–VDAC2 axis) to fully appreciate how they can use a single mechanism to yield different cellular phenotypes. For example, a potentially prosurvival mechanism characterized by permeabilization of the peroxisome membrane to release catalase greatly contrasts with committing a cell to the mitochondrial pathway of apoptosis by promoting cytochrome *c* release (Fig. 1). Given the number of proteins and pathways associated with the organelles described here, years of investigation will likely follow to fully integrate these observations into a more complete understanding of organelle biology and human disease.

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