

# Caspase-8 goes cardiolipin: a new platform to provide mitochondria with microdomains of apoptotic signals?

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In certain cell types, apoptosis in response to extracellular stimuli like Fas depends on a mitochondrial amplificatory loop: the apical caspase-8 cleaves and activates the BH3-only member of the Bcl-2 family BID. In turn, BID induces the release of cytochrome *c* from mitochondria to the cytoplasm, where it is required to fully activate effector caspases. In this issue of *The Journal of Cell Biology*, Gonzalez et al. (see p. 681) show that when caspase-8 activation and production of functional BID is required, it is performed on mitochondrial platforms provided by the mitochondrion-specific lipid cardiolipin. Cardiolipin anchors caspase-8 at contact sites between inner and outer mitochondrial membranes, facilitating its self activation. These findings suggest that like other second messengers such as  $\text{Ca}^{2+}$  and cAMP, production of apoptotic messengers can be compartmentalized in close proximity to their intracellular target.

The developmental and homeostatic control over cell number and type in tissues and organs is accomplished by the tight equilibrium between proliferation and apoptosis. Old, damaged, unnecessary cells are eliminated by a genetically defined program of self destruction that, under many circumstances, ensues from an extracellular stimulus that engages a death receptor on the surface of the target cell and triggers an intracellular “killing” cascade (Peter and Kramer, 2003). Death caused from within as well as from the outside share the executioners, the effector caspases. However, how efficient activation of these proteases is accomplished differs between the two cases. In response to the activation of a death receptor, a platform (the so called death-induced signaling complex [DISC]) is assembled under the plasma membrane where the proenzymes of the apical caspases, like procaspase-8, are recruited and undergo activation by proximity. Their self cleavage is autoamplificatory in nature,

and when enough DISCs are formed, the concentration of active apical caspases in the cell reaches levels high enough to directly process and turn effector caspases on (Peter and Kramer, 2003). When, conversely, the amount of active caspase-8 is lower, additional amplificatory mechanisms are required. In these cell types, called type II, caspase-8 cleaves and activates a BH3-only member of the Bcl-2 family of proteins, triggering its translocation to mitochondria, where it causes the release of cytochrome *c* (Scaffidi et al., 1998). In this issue, Gonzalez et al. (see p. 681) show a novel, unexpected role for mitochondria and for a mitochondrial lipid in particular, cardiolipin (CL), in the activation of the apical caspase-8 in cells that require the mitochondrial amplificatory loop. When type II cells are triggered to die by extrinsic stimuli, they end up using mitochondria as their favorite surface to generate cleaved BID. Caspase-8 translocates to mitochondria, where it binds to CL to become activated by proximity, leading to the proteolytically active p43 and p10 fragments. These are inserted into the outer mitochondrial membrane and form an oligomer that can be stabilized on the membrane. Gonzalez et al. (2008) demonstrate that this association with mitochondria depends on the presence of mature CL, which proves essential *in situ* and *in vitro* for the insertion of caspase-8 into natural and artificial membranes. The final outcome is that the production of active, cleaved BID is accomplished where it is needed, i.e., on the surface of its target organelle (Fig. 1).

The BH3-only subset of the Bcl-2 family comprises a large number of molecules (like BAD, BIM, NOXA, PUMA, and BIK) that, in response to intrinsic or extrinsic death stimuli, transduce the signal to the multidomain proapoptotics, like BAK and BAX, whose activation results in the release of proapoptotic factors, like cytochrome *c*, from mitochondria (Scorrano and Korsmeyer, 2003). Cytochrome *c* is the only soluble component of the mitochondrial respiratory chain, and, once in the cytoplasm, it complexes with the adaptor Apaf-1 and the pro-caspase 9 to cause activation and self cleavage of the latter, an extremely efficient process (Wang, 2001). Normally, the vast majority

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the surface of mitochondria via specific AKAP binding proteins suggests the existence of cAMP microdomains on the surface of the organelle (Chen et al., 1997). Finally, biosynthesis of mitochondrial lipids requires a structural compartmentalization provided by the mitochondria-associated membranes, areas of close juxtaposition with the endoplasmic reticulum where several steps of the biosynthesis occur (Daum and Vance, 1997). What could be the functional consequences of a compartmentalized production of BID on the surface of mitochondria? This could avoid BID being “mistargeted” to other intracellular membranes, such as endoplasmic reticulum (Gajkowska et al., 2004); alternatively, the production of high locales of BID at contact sites allows it to insert in the inner membrane and trigger cristae remodeling (Kim et al., 2004). Finally, compartmentalization could be required to place BID in proximity to specific mitochondrial ubiquitin ligases (like MARCH-5, for example, Karbowski et al., 2007) because BID activation requires degradation of the N terminus after cleavage by caspase-8 (Tait et al., 2007).

The work of Gonzalez et al. (2008) leaves the question of the pathogenesis of Barth’s syndrome open. Mutations in *Tafazzin* that alter the total amount of mature CL in mitochondria cause a resistance in Fas-induced apoptosis and basically do not affect the death induced by intrinsic stimuli. So, what is the underlying mechanism for the observed myopathy and neutropenia? One simple possibility is that cells lacking mature CL display mitochondrial dysfunction, as it was observed in yeast (Ma et al., 2004). Alternatively, the block in apoptosis caused by the lack of mature CL might trigger an excess of autophagy that ultimately causes the clinical phenotype. Autophagy has indeed been described in neutropenia and cardiomyopathy, and it is known that blockage of the mitochondrial death pathway, in the long run, causes death by autophagy (Shimizu et al., 2004). Future research on the pathogenesis of Barth syndrome will surely capitalize on the work by Gonzalez et al. (2008) and look into these possible new directions.

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## References

Ardaill, D., J.P. Privat, M. Egret-Charlier, C. Levrat, F. Lerme, and P. Louisot. 1990. Mitochondrial contact sites. Lipid composition and dynamics. *J. Biol. Chem.* 265:18797–18802.

Barth, P.G., R.J. Wanders, P. Vreken, E.A. Janssen, J. Lam, and F. Baas. 1999. X-linked cardioskeletal myopathy and neutropenia (Barth syndrome) (MIM 302060). *J. Inherit. Metab. Dis.* 22:555–567.

Chen, Q., R.Y. Lin, and C.S. Rubin. 1997. Organelle-specific targeting of protein kinase AII (PKAII). Molecular and in situ characterization of murine A kinase anchor proteins that recruit regulatory subunits of PKAII to the cytoplasmic surface of mitochondria. *J. Biol. Chem.* 272:15247–15257.

Danial, N.N., C.F. Gramm, L. Scorrano, C.Y. Zhang, S. Krauss, A.M. Ranger, S.R. Datta, M.E. Greenberg, L.J. Licklider, B.B. Lowell, et al. 2003. BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis. *Nature.* 424:952–956.

Daum, G., and J.E. Vance. 1997. Import of lipids into mitochondria. *Prog. Lipid Res.* 36:103–130.

Frezza, C., S. Cipolat, O. Martins de Brito, M. Micaroni, G.V. Beznoussenko, T. Rudka, D. Bartoli, R.S. Polishuck, N.N. Danial, B. De Strooper, and L. Scorrano. 2006. OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. *Cell.* 126:177–189.

Gajkowska, B., U. Wojewodzka, and J. Gajda. 2004. Translocation of Bax and Bid to mitochondria, endoplasmic reticulum and nuclear envelope: possible control points in apoptosis. *J. Mol. Histol.* 35:11–19.

Gonzalez, F., Z.T. Schug, R.H. Houtkooper, E.D. MacKenzie, D.G. Brooks, R.J.A. Wanders, P.X. Petit, F.M. Vaz, and E. Gottlieb. Cardiolipin provides an essential activating platform for caspase-8 on mitochondria. *J. Cell Biol.* 183:681–696.

Karbowski, M., A. Neutzner, and R.J. Youle. 2007. The mitochondrial E3 ubiquitin ligase MARCH5 is required for Drp1-dependent mitochondrial division. *J. Cell Biol.* 178:71–84.

Kim, T.H., Y. Zhao, W.X. Ding, J.N. Shin, X. He, Y.W. Seo, J. Chen, H. Rabinowich, A.A. Amoscato, and X.M. Yin. 2004. Bid-cardiolipin interaction at mitochondrial contact site contributes to mitochondrial cristae reorganization and cytochrome C release. *Mol. Biol. Cell.* 15:3061–3072.

Kuwana, T., M.R. Mackey, G. Perkins, M.H. Ellisman, M. Latterich, R. Schneider, D.R. Green, and D.D. Newmeyer. 2002. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. *Cell.* 111:331–342.

Lutter, M., M. Fang, X. Luo, M. Nishijima, X. Xie, and X. Wang. 2000. Cardiolipin provides specificity for targeting of tBid to mitochondria. *Nat. Cell Biol.* 2:754–761.

Ma, L., F.M. Vaz, Z. Gu, R.J. Wanders, and M.L. Greenberg. 2004. The human TAZ gene complements mitochondrial dysfunction in the yeast taz1Delta mutant. Implications for Barth syndrome. *J. Biol. Chem.* 279:44394–44399.

Ott, M., J.D. Robertson, V. Gogvadze, B. Zhivotovsky, and S. Orrenius. 2002. Cytochrome c release from mitochondria proceeds by a two-step process. *Proc. Natl. Acad. Sci. USA.* 99:1259–1263.

Peter, M.E., and P.H. Krammer. 2003. The CD95 (APO-1/Fas) DISC and beyond. *Cell Death Differ.* 10:26–35.

Rizzuto, R., M. Brini, M. Murgia, and T. Pozzan. 1993. Microdomains with high Ca<sup>2+</sup> close to IP<sub>3</sub>-sensitive channels that are sensed by neighboring mitochondria. *Science.* 262:744–747.

Scaffidi, C., S. Fulda, A. Srinivasan, C. Friesen, F. Li, K.J. Tomaselli, K.M. Debatin, P.H. Krammer, and M.E. Peter. 1998. Two CD95 (APO-1/Fas) signaling pathways. *EMBO J.* 17:1675–1687.

Scorrano, L., and S.J. Korsmeyer. 2003. Mechanisms of cytochrome c release by proapoptotic BCL-2 family members. *Biochem. Biophys. Res. Commun.* 304:437–444.

Shimizu, S., T. Kanaseki, N. Mizushima, T. Mizuta, S. Arakawa-Kobayashi, C.B. Thompson, and Y. Tsujimoto. 2004. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. *Nat. Cell Biol.* 6:1221–1228.

Tait, S.W., E. de Vries, C. Maas, A.M. Keller, C.S. D’Santos, and J. Borst. 2007. Apoptosis induction by Bid requires unconventional ubiquitination and degradation of its N-terminal fragment. *J. Cell Biol.* 179:1453–1466.

Wang, X. 2001. The expanding role of mitochondria in apoptosis. *Genes Dev.* 15:2922–2933.

Xu, Y., A. Malhotra, M. Ren, and M. Schlame. 2006. The enzymatic function of tafazzin. *J. Biol. Chem.* 281:39217–39224.