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# Death-Defying Yeast Identify Novel Apoptosis Genes

Minireview

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Intensive study of processes that initiate, control, and execute the cell death program termed apoptosis has resulted in a wealth of new information regarding how cells contribute to their own demise. The picture emerging from these studies suggests a complex interplay between factors that promote cell death and those that prevent cell death, the end result being life or death of the cell (for reviews, see Horvitz et al., 1994; Salvesen and Dixit, 1997, and references therein). The most downstream components of the cell-death machinery identified so far are proteases known as caspases, a class of cysteine proteases that cleave substrates following aspartate residues. Most apoptotic processes, from those in the nematode *Caenorhabditis elegans* to those in humans, result in activation of these proteases leading to cell death (Figure 1). The activity of caspases can be regulated by a variety of cellular factors. Some, such as the *C. elegans* protein CED-4, the related mammalian protein Apaf-1, or the mammalian protein Fas, can activate caspases and are thus death promoters (Shaham and Horvitz, 1996; Salvesen and Dixit, 1997; Zou et al., 1997). Others, such as *C. elegans* CED-9 or mammalian Bcl-2 can inhibit activation of caspases. Yet other proteins, such as mammalian Bax, which is similar to Bcl-2, seem to promote cell death in part by inhibiting the activities of death-preventing proteins of the Bcl-2 family. Regulators such as Bax can, in turn, be inhibited by Bcl-2 (Korsmeyer et al., 1993) (Figure 1).

Although some of the interactions among known cell-death components have been described, there are still major gaps in our understanding of the apoptotic death process. For example, the cellular targets of caspases that lead to cell death have not yet been fully described. In addition, the mechanisms by which death-preventing members of the Bcl-2 family inhibit caspase activation and how Bax and other death-promoting relatives of Bcl-2 promote cell death also remain obscure.

Two papers published in the current issue of *Molecular Cell* (Xu and Reed, 1998; Matsuyama et al., 1998) provide a novel entry to understanding how Bax and Bcl-2 might function to promote and prevent apoptosis, respectively. The authors describe the identification of two new components of the cell-death pathway using the budding yeast *Saccharomyces cerevisiae*. Although budding yeast does not exhibit apoptosis or contain homologs of Bcl-2 or caspases, Bax and a closely related death-promoting protein, Bak, have been previously described to induce cell death both in this organism and in fission yeast. These deaths can be inhibited by coexpression of Bcl-2. Furthermore, expression of mutant versions of Bax or Bak that lack activity in mammalian cells do not result in yeast lethality (Zha et al., 1996; Ink et al., 1997). These observations suggest that the same properties that cause Bax to kill mammalian cells are required for it to kill yeast cells. Using a Bax gene whose expression is induced in the presence of galactose (pGal-Bax), Xu and Reed (1998) and Matsuyama et al. (1998) searched for human cDNAs and yeast mutations, respectively, that prevent Bax-induced cell death in galactose-containing medium. Their results support the idea that yeast can be used as a vehicle for identifying metazoan cell-death factors.

### *Bax Inhibitor-1 (BI-1) Inhibits Mammalian Apoptosis*

Xu and Reed (1998) transformed pGal-Bax-containing yeast cells with a human cDNA library in which cDNAs were fused to a constitutively active yeast promoter and isolated four cDNAs that prevent lethality on galactose. Three encoded the same protein, termed BI-1 (for **B**ax **I**nhibitor-**1**) and were studied further. The *BI-1* gene is identical to a previously isolated human gene of unknown function called *TEGT* (**T**estis **E**nhanced **G**ene **T**ranscript). Homologs of BI-1 are found in both rat and mouse as well as in the plant *Arabidopsis thaliana*. Weak similarity was also found with a *C. elegans* gene, indicating that BI-1 is conserved in evolution.

To determine if BI-1 plays a role in mammalian apoptosis, the authors tested the ability of BI-1 to block cell death induced by Bax overexpression, growth factor deprivation, etoposide, and staurosporine treatment of cultured mammalian cells. They found that BI-1 could inhibit cell death to a similar extent as Bcl-2, suggesting that BI-1 might normally function to prevent mammalian apoptosis. Expression of antisense BI-1 RNA induced apoptosis in approximately 20% of cultured 293 cells, supporting the notion that BI-1 is a mammalian cell-death inhibitor. Evidence that BI-1 is a key regulator of mammalian apoptosis could be supplied by analysis of BI-$1^{-/-}$ mice. Increased apoptosis in such knockout mice would provide strong support for the importance of BI-1 as an inhibitor of apoptosis.

The observation that BI-1 inhibits Bax-induced death in both mammalian cells and *S. cerevisiae* is remarkable. These results are a clear validation of the approach used to identify genes affecting Bax activity. The screen used by the authors does not appear to have been exhaustive,

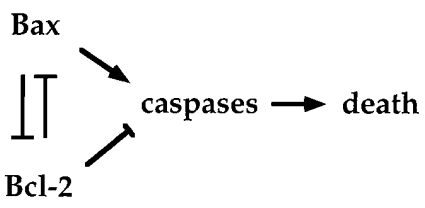


Figure 1. Regulation of Caspases and Cell Death by Bax and Bcl-2

Blunt arrows indicate inhibition. Pointed arrows indicate activation. Some reports suggest that Bax and Bcl-2 can also promote and prevent cell death, respectively, in a caspase-independent manner (see, for example, Xiang et al., 1996).

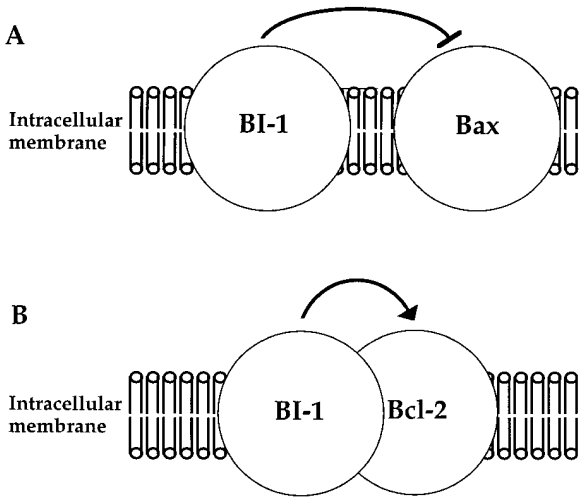


Figure 2. Possible Models for the Function of BI-1

(A) BI-1 might not directly associate with Bax, a death-promoting protein, yet still inhibit Bax activity, thus preventing cell death.
(B) BI-1 could directly associate with and activate Bcl-2, a death-preventing protein, to prevent cell death.

since one cDNA class isolated was represented by only a single cDNA. Furthermore, at least one gene that should have been isolated, namely *Bcl-2* (Figure 1), was not recovered. Thus, continued screening would likely reveal additional cell-death inhibitors.

The *BI-1* gene encodes a protein containing several putative transmembrane domains. Examination of BI-1 localization using GFP- or FLAG-tagged BI-1 revealed that these proteins are located primarily in the endoplasmic reticulum (ER) membrane and nuclear envelope. Weak staining was also detected in mitochondrial membranes. Interestingly, Bcl-2 had been previously localized to intracellular membranes (reviewed by Kroemer, 1997). Cell fractionation experiments performed by the authors reveal that tagged BI-1 and Bcl-2 colocalize, suggesting that these proteins might interact. Support for this notion was provided by both in vivo cross-linking and coimmunoprecipitation experiments.

How might BI-1 prevent cell death in mammalian cells? Based on the general scheme shown in Figure 1, the primary action of BI-1 might be either to inhibit Bax or to stimulate Bcl-2 (Figure 2). If BI-1 inhibits Bax activity, it is unlikely to do so by direct association since the two proteins do not coimmunoprecipitate (Figure 2A). BI-1 could inhibit Bax activity without direct association, for example, by activating an inhibitor of Bax. The ability of BI-1 to associate with Bcl-2 in vivo suggests that Bcl-2 could be such an inhibitor. Alternatively, BI-1 could activate Bcl-2 to inhibit death in a Bax-independent manner (Figure 2B). Whether BI-1 activates Bcl-2 could be tested by determining whether overexpression of BI-1 prevents the death of cells lacking Bcl-2.

Although overexpression of BI-1 prevented cell death induced by growth factor depletion and other conditions (see above), it was unable to prevent apoptosis resulting from activation of Fas, a cell-surface receptor that promotes cell death when activated. The Fas receptor is thought to promote cell death by activating a cascade of caspases and can function in a Bcl-2-independent manner (Vaux and Strasser, 1996; Salvesen and Dixit, 1997). BI-1 is thus not a universal cell death inhibitor and apparently affects some modes of apoptosis but not others.

### *Mitochondrial $F_0F_1$-ATPase—a Possible Role in Bax-Induced Apoptosis*

In order to identify components required for Bax-induced cell death in yeast, Matsuyama et al. (1998) screened for yeast mutants that do not exhibit pGal-Bax-induced death on galactose-containing medium. The authors isolated one recessive Bax-resistant yeast mutant and cloned the relevant gene by standard methods of genetic complementation. The gene (*ATP4*) encodes a subunit of the yeast $F_0F_1$-ATPase, a proton pump required for aerobic respiration that is located in the inner mitochondrial membrane. Interestingly, recent evidence has suggested an important role for mitochondria in some forms of apoptosis. Mitochondrial cytochrome c can be detected in the cytoplasm of mammalian cells undergoing apoptosis, and, in association with Apaf-1, a protein related to *C. elegans* CED-4, can activate certain caspases in vitro (Zou et al., 1997). In addition, several members of the Bcl-2 family including Bcl-2 and Bax have been shown to associate with the outer mitochondrial membrane in mammalian cells. Overproduction of Bcl-2 can prevent both cytochrome c release and apoptosis in mammalian cells (reviewed by Reed, 1997). When overexpressed in yeast, Bax is primarily associated with the outer mitochondrial membrane and can also cause cytochrome c release (Manon et al., 1997), suggesting that Bax-induced death in yeast might share mechanistic similarities with mammalian apoptosis.

A variety of observations support the argument that the $F_0F_1$-ATPase is required for Bax-induced killing in yeast. First, Matsuyama et al. demonstrated that interruption of the *ATP4* coding sequence also suppressed Bax-mediated killing, thus confirming the results of their screen. Second, a mutation in a different subunit of the $F_0F_1$-ATPase also inhibited Bax-induced killing. Third, yeast cells exposed to oligomycin, a potent inhibitor of the $F_0F_1$-ATPase, did not undergo Bax-induced death. Fourth, mutant yeast cells unable to respire via mitochondrial oxidative phosphorylation yet containing an intact $F_0F_1$-ATPase were not protected against Bax killing. These experiments strongly suggest that the activity of the $F_0F_1$-ATPase, and probably not respiration in general, is required for killing of yeast cells by Bax.

As with the paper by Xu and Reed (1998), Matsuyama et al. tested the relevance of their discoveries in mammalian cells, where Bax normally acts. Strikingly, the authors showed that death of mammalian cells induced by Bax could be partially inhibited by oligomycin but not by antimycin, a drug affecting mitochondrial respiration. Caspase activation during Bax-induced cell death was also inhibited by oligomycin. These results mirror the yeast experiments and suggest that Bax activity in mammalian cells may at least partially require a functional $F_0F_1$-ATPase. It would be interesting to test if the $F_0F_1$-ATPase is required for cell death other than that induced by Bax in both mammals and yeast. If so, this protein complex might define a key step of the cell death process.

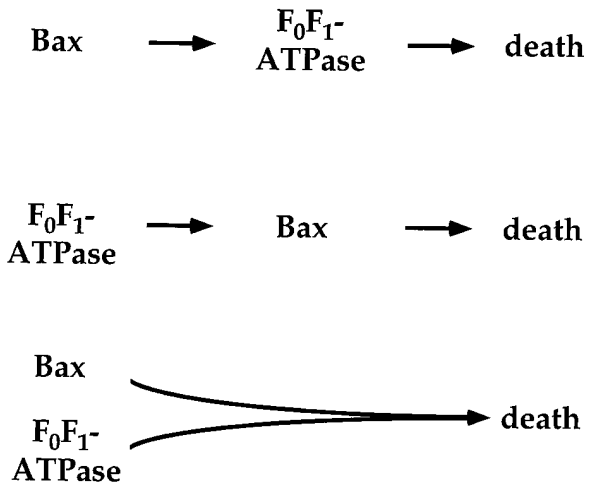


Figure 3. Possible Interactions between Bax and the $F_0F_1$-ATPase in Mammals and Yeast

Bax could act either upstream (top), downstream (middle), or in parallel (bottom) to the $F_0F_1$-ATPase to promote cell death.

The $F_0F_1$-ATPase might promote Bax-induced killing in mammals and in yeast by activating Bax, by being a target of Bax activity, or by acting in parallel to Bax (Figure 3). The data presented by the authors do not allow discrimination among these possibilities. Bax and other Bcl-2 family members have been shown to form pores when inserted into membranes (Schlesinger et al., 1997, and references therein). It is thus possible that the interaction between Bax and the $F_0F_1$-ATPase, a proton pump, reflects a role for ion transporters in apoptosis. The $F_0F_1$-ATPase is localized to the inner mitochondrial membrane. Intriguingly, cytochrome c is located in the intermembrane space of mitochondria and can interact with some proteins of the mitochondrial inner membrane. Thus, if cytochrome c release into the cytoplasm plays a causal role in mammalian and yeast cell death, then the $F_0F_1$-ATPase might be necessary for this release.

### *Yeast and Apoptosis*

The requirement for the $F_0F_1$-ATPase in Bax-induced death of yeast cells suggests that yeast may contain additional conserved components of an apoptotic pathway. Reports in the literature suggest that death of either budding or fission yeast in response to overexpression of Bak or mutation of the budding yeast *CDC48* gene result in chromatin condensation, DNA degradation and membrane blebbing—cellular features that are hallmarks of metazoan apoptosis (Ink et al., 1997; Madeo et al., 1997). In addition, Bcl-2 has been reported to improve viability of yeast mutants defective in *SOD1* under some growth conditions. *SOD1* codes for the cytoplasmic Cu-Zn superoxide dismutase, which functions in detoxifying free radicals. These results are similar to those in mammalian cells and suggest that Bcl-2 might have antioxidant properties (Long et al., 1997). It is thus possible that molecular pathways leading to certain subphenotypes of apoptotic cells are present in yeast cells. Conservation of a number of functional modules between yeast and mammals is certainly striking. Yeast cells harbor modules such as MAP kinase cascades, GTPases and their exchange factors, and G proteins and their regulators, which have shed light on analogous mammalian processes even though the components may be used in different functional contexts. Similar modules might exist in metazoan apoptotic pathways.

Even though yeast does not contain obvious homologs of known metazoan cell-death regulators, there are a variety of strategies that could be used to look for yeast products involved in yeast cell death. Proteins that inhibit cell death (BI-1 analogs) could be identified by looking for yeast genes whose overexpression inhibits Bax-mediated killing. Such proteins could also be discovered by looking for genes whose inactivation results in lethality that is suppressed by expression of a metazoan death inhibitor such as Bcl-2. Conserved components of a cell death pathway (Bax analogs) could also be identified by screening for yeast genes whose expression kills yeast cells in an $F_0F_1$-ATPase-dependent manner. Finally, experiments similar to those described in Xu and Reed (1998) and Matsuyama et al. (1998) could be carried out on yeast cells induced to die by expression of metazoan death promoters other than Bax.

The current picture of apoptosis has been developed from studies of a variety of organisms—nematodes, flies, and animal cells. The papers reviewed here suggest that using yeast to study apoptosis might be richly rewarding. Studying metazoan cell death in yeast may thus turn into a major growth (death) industry.

#### Selected Reading

Horvitz, H.R., Shaham, S., and Hengartner, M.O. (1994). Cold Spring Harb. Symp. Quant. Biol. *59*, 377–385.

Ink, B., Zöring, M., Baum, B., Hajimagheri, N., James, C., Chittenden, T., and Evan, G. (1997). Mol. Cell. Biol. *17*, 2468–2474.

Korsmeyer, S.J., Shutter, J.R., Veis, D.J., Merry, D.E., and Oltvai, Z.N. (1993). Semin. Canc. Biol. *4*, 327–332.

Kroemer, G. (1997). Nature Med. *3*, 614–620.

Longo, V.D., Ellerby, L.M., Bredesen, D.E., Valentine, J.S., and Gralla, E.B. (1997). J. Cell Biol. *137*, 1581–1588.

Madeo, F., Fröhlich, E., and Fröhlich, K.-U. (1997). J. Cell Biol. *139*, 729–734.

Manon, S., Chaudhuri, B., and Buérin, M. (1997). FEBS Lett. *415*, 29–32.

Matsuyama, S., Xu, Q., Velours, J., and Reed, J.C. (1998). Mol. Cell *1*, 327–336.

Reed, J.C. (1997). Cell *91*, 559–562.

Salvesen, G.S., and Dixit, V.M. (1997). Cell *91*, 443–446.

Schlesinger, P.H., Gross, A., Yin, X.M., Yamamoto, K., Saito, M., Waksman, G., and Korsmeyer, S.J. (1997). Proc. Natl. Acad. Sci. USA *94*, 11357–11362.

Shaham, S., and Horvitz, H.R. (1996). Genes Dev. *10*, 578–591.

Vaux, D.L., and Strasser, A. (1996). Proc. Natl. Acad. Sci. USA *93*, 2239–2244.

Xiang, J., Chao, D.T., and Korsmeyer, S.J. (1996). Proc. Natl. Acad. Sci. USA *93*, 14559–14563.

Xu, Q., and Reed, J.C. (1998). Mol. Cell *1*, 337–346.

Zha, H., Fisk, H.A., Yaffe, M.P., Mahajan, N., Herman, B., and Reed, J.C. (1996). Mol. Cell. Biol. *16*, 6494–6508.

Zou, H., Henzel, W.J., Liu, X., Lutschg, A., and Wang, X. (1997). Cell *90*, 405–413.