Modulation of Cell Death in Yeast by the Bcl-2 Family of Proteins*

Weikang Tao‡§, Cornelia Kurschner‡, and James I. Morgan‡¶

From the ‡Department of Developmental Neurobiology, St. Jude Children’s Research Hospital, Memphis, Tennessee 38105 and the §Department of Biochemistry and Molecular Biology, University of Medicine and Dentistry of New Jersey-Graduate School of Biochemical Sciences, Newark, New Jersey 07103

Bcl-2 family members are regulators of cell death. The precise biochemical properties of these proteins are unclear although intrafamily protein-protein association is thought to be involved. To elucidate structure-activity relationships among Bcl-2 proteins and identify the pathways in which they act, an inducible death suppressor assay was developed in yeast. Only Bax and Bak killed yeast via a process that did not require interleukin-1β-converting enzyme-like proteases. Bax/Bak lethality was suppressed by coexpression of Bcl-2 family members that are anti-apoptotic in vertebrates, namely Bcl-xL, Bcl-2, Mcl-1, and A1. Furthermore, Bcl-xL and Bcl-2 suppressed Bax toxicity by distinct mechanisms in yeast. Bad, Bcl-xS, and Ced-9 lacked suppressor activity. These inactive proteins bound to anti-apoptotic members of the Bcl-2 family but not to Bax or Bak. In contrast, most Bcl-2 family proteins that attenuated death bound to Bax and Bak. However, two mutants of Bcl-xL suppressed Bax-induced cell death while having no Bax binding activity. Therefore, Bcl-xL functions independently of Bax binding, perhaps by interacting with a common target or promoting a pathway that antagonizes Bax. Thus, the pathways downstream of Bax and Bcl-xL may be conserved between vertebrates and yeast. This suppressor assay could be used to isolate components of these pathways.

Cell death is a highly regulated process involving interactions among extracellular molecules, intracellular signal transduction pathways, and resident suicide/rescue programs (1, 2). Studies in Caenorhabditis elegans have pointed to a cell suicide pathway that includes several molecules that have homologs in vertebrates (3). Central among these death-regulating proteins is Ced-9, which suppresses programmed cell death in C. elegans (4). Bcl-2, the vertebrate homolog of Ced-9, was identified independently through its translocation in many B cell follicular lymphomas in man (5, 6). Bcl-2 inhibits cell death in various circumstances in vertebrate cells and functionally substitutes for Ced-9 in C. elegans (7, 8). Subsequent investigations have identified a number of proteins in vertebrates that are structurally related to Bcl-2 (7). These proteins constitute a family, the members of which share a number of regions of homology, termed BH1 (Bcl-2 homology), BH2, and BH3 domains (9, 10). Some of these Bcl-2 related proteins, such as Bcl-xL, also prevent cell death, whereas others, such as Bax, provoke cell elimination. The biochemical and biophysical mechanisms that confer these properties on the Bcl-2 family of proteins remain enigmatic, although recent structural data suggest that they may be pore-forming proteins (11). However, Bcl-2 and many of its related proteins can participate in homo- and heteromeric complexes, and it has been suggested that the activity of pro-apoptotic members of the Bcl-2 family is neutralized by their association with anti-apoptotic members (12, 13).

There are a number of caveats in the interpretation of the role of Bcl-2 family members in the regulation of cell death in vertebrates. First, the effects of Bcl-2 family members are often assessed in models where cell death is triggered by an exogenous means such as growth factor withdrawal or addition of a toxin or virus to the culture medium. While this is more relevant to the physiological situation, it adds a level of ambiguity as to whether the effects are mediated through intrafamily interactions. Second, a given cell type may already express the gene of interest as well as other known and potentially unknown members of the Bcl-2 family. Thus, there is uncertainty as to precisely which proteins interact to produce the observed effect. Finally, Bcl-2 family members are differentially expressed, and bcl-2- and bcl-x-null mice have distinct phenotypes (14, 15). Together, these data imply that the various Bcl-2-like proteins have functional differences. Indeed, there are indications that Bcl-xL need not dimerize with Bax to suppress cell killing and that Bcl-2 and Bcl-xL have differential activities in some assays (14–18). Thus, from the mechanistic standpoint, there is a need for a model in which the role of Bcl-2 family members in cell death can be determined without the foregoing ambiguities.

Recently, several studies reported that the expression of Bax is lethal in the budding yeast, Saccharomyces cerevisiae (19–21). This is despite the fact that yeasts express no identifiable members of the Bcl-2 family and are not known to undergo programmed cell death. This afforded the opportunity to develop a suppressor assay in which the ability of Bcl-2 family members to attenuate Bax killing could be determined quantitatively. Moreover, since the model is in essence the same as a two-hybrid system, protein-protein association can be assessed simultaneously.

It is shown that Bcl-2, Bcl-xL, Mcl-1, and A1 can suppress death induced by Bax and Bak in yeast, whereas Ced-9, Bad, and Bcl-xS are inactive. Bcl-2 and Bcl-xL have differential suppression activities in some assays (14–18). Thus, from the mechanistic standpoint, there is a need for a model in which the role of Bcl-2 family members in cell death can be determined without the foregoing ambiguities.
Structure-Activity Relationships of Bcl-2 Family in Yeast

EXPERIMENTAL PROCEDURES

Yeast Strains, Growth, and Transformation—The S. cerevisiae strain S260 (ura3 trp1) contained a genomic LEXA-operator-LACZ fusion reporter gene and was described previously (22). Yeast growth, maintenance, and transformations were as described (23).

Yeast Expression Constructs—Fusion proteins with the LexA DNA binding domain were constructed in the yeast expression plasmid, Y.LexA (22), which carries the S. cerevisiae TRP1 gene as a selectable marker. Fusion proteins with the VP16 transcriptional activation domain were generated in pSD.10a, which harbors the marker. Fusion proteins with the VP16 transcriptional activation domain were generated in pSD.10a, which harbors the URA3 selection marker (24). Constructs lacking heterologous fusion sequences were made in pSD.10a after deletion of the VP16 codos.

LexA and VP16 fusions of murine Bax, Bcl-2, and A1 were reported previously (22). cDNAs encoding murine Bcl-x, Bak, and Bad were obtained by reverse transcription polymerase chain reaction using mouse brain RNA and polymerase chain reaction primers based upon obtained by reverse transcription polymerase chain reaction using mouse brain RNA and polymerase chain reaction primers based upon published sequences. Murine mcl-1 cDNA was isolated in a yeast two-hybrid screening for Bax-binding proteins. A Ced-9 cDNA was a gift from Dr. D. Pickup. Truncation and deletion mutants were generated by polymerase chain reaction. The sequences of all constructs were verified.

In Vitro Translation Constructs—For in vitro translation/translation reactions, cDNAs encoding full-length Bcl-xL and two of its mutants (XF14 and XF15) were inserted into the vector pT7/plink (24). A full-length murine bcl-2 cDNA was cloned into the pT7/plink-TaqN (22). This construct, an epitope tag derived from human c-MYC protein is fused to the N terminus of Bax. This epitope is recognized by the monoclonal antibody 9E10.

Complementation—Proteins were translated in vitro as described (22). MYC-tagged Bax was translated in the absence of [35S]methionine (Amersham Life Science, Inc.), and untagged Bcl-xL and its mutants XF14 and XF15 were translated in the presence of [35S]methionine. For complementation studies, 2 µl of the Bax-MYC translation reaction were mixed with 10 µl of the Bcl-xL, XF14, or XF15 translation reactions, respectively. Incubation and washing steps were performed as described (26) in NETgel buffer with 0.2% Nonidet P-40. The precipitating antibody was 9E10 (Santa Cruz Biotechnology, Inc.). Protein A-Sepharose CL-4B (Sigma) was used to precipitate the immune complexes.

Yeast Two-hybrid Analysis—S260 was transformed with two plasmids encoding a LexA fusion construct and a VP16 hybrid. Transformants were grown and assayed for β-galactosidase activity as described (22). The development of blue color in the yeast colonies was monitored for 24 h.

Yeast Growth Assay—S260 was cotransformed with expression plasmids encoding Bcl-2 family members. Selective media containing 2% glucose was inoculated with a single colony of transformants and incubated overnight at 30 °C. Subsequently, cells were washed three times with H2O. Typically, 20 ml of selective medium (with 2% galactose) was inoculated with 2.56 × 106 cells, and incubation was continued. Samples were taken at different time points, and cell density was measured by determining the OD at 660 nm. To compare results from different experiments, a growth index (GI) was devised. (OD660/0 h − OD660/24 h) (cells containing Bax or Bak and the test protein)/(OD660/0 h − OD660/24 h) (cells containing Bax or Bak and LexA or VP16)

Immunoblotting—Yeast cells were lysed mechanically as described (26). Proteins were separated on 15% SDS-polyacrylamide gels (30 µg/lane) and transferred to nitrocellulose membranes. Immunostaining was performed in Tris-buffered saline containing 1% fetal calf serum at room temperature. Membranes were incubated with a rabbit anti-murine Bax polyclonal antibody, 13666E (1:1000 dilution) (Pharmin- gen), followed by horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin antisera (1:1000) (Amersham, both for 2 h. Immuno- blots were developed using dianaminobenzidine as chromogen (27). For Bcl-xL immunostaining, the membrane was stripped of bound antibody, reprobed with a monoclonal anti-Bcl-x antibody, B22620 (1:250) (Transduction Laboratories). The immunoblots were developed using the enhanced chemiluminescence method (Amersham).

RESULTS

Identification of Members of the Bcl-2 Family That Are Le- thal in Yeast—Since Bax kills yeast (19–21), additional members of the Bcl-2 family (Bcl-2, Bcl-xL, Bcl-xS, A1, Mcl-1, Bad, Bak, and Ced-9) were tested for this property. Besides Bax, only Bak was lethal, although it was consistently less potent (Table I). Moreover, like native Bax, the LexA and VP16 fusion proteins of Bak and Bak used in the two-hybrid assay were also lethal (Fig. 1, A and B, and Table I), making it possible to correlate binding activity with biological activity in subsequent studies.

Suppression of Bax- and Bak-induced Lethality by Bcl-2 Family Members—To quantitatively determine the suppression of Bax/Bak toxicity in yeast, cDNAs encoding LexA or VP16 fusions of various Bcl-2 family members were coexpressed with either Bax or Bak. Coexpression of the Bcl-xL fusion proteins generated for the two-hybrid system inhibited Bax and Bak toxicity (Fig. 1A and Table I). In addition, LexA fusions of Bcl-2, Mcl-1, and A1 inhibited both Bax and Bak killing (Fig. 2 and Table I). Bcl-xS, Bad, and Ced-9 did not attenuate Bax or Bak lethality (Table I). To ensure that the results were not artifacts of LexA or VP16 fusion proteins, these data were confirmed using native (unfused) protein se- quences. Both native Bcl-2 (GI, 7.4) and native Bcl-xL (GI, 8.0) suppressed the killing elicited by native Bax (GI, 1.0).

Relationship between the Anti-death Activity of Bcl-2 Family Members and Their Binding to Bax and Bak—In the yeast two-hybrid assay, Bcl-xL, Bcl-2, Mcl-1, and A1 all bound to Bax and Bak, whereas Bcl-xS, Bad, and Ced-9 did not (Table I). Therefore, anti-death activity was associated with the ability to bind to Bax or Bak. Proteins such as Ced-9, Bcl-xS, and Bad that did not bind to Bax/Bak did not inhibit killing, whereas proteins that could associate, such as Bcl-xL and Mcl-1, were inhibitory. Since the lack of death suppressor activity of Ced-9 was unexpected, its association with anti-apoptotic members of the Bcl-2 family was determined. LexA-Ced-9 (as well as LexA- Bcl-xS and LexA-Bad) bound to Bcl-xL, Bcl-2, and A1 (data not shown). Therefore, Ced-9 had the binding and activity profiles of proteins such as Bcl-xS rather than Bcl-2.

Mutations in Bcl-xL Dissociate Bax Binding from Death Sup- pressor Activity—Further to pursue the relationship between heterodimerization and biological activity, a series of trunca- tion and deletion mutations of Bcl-2 and Bcl-xL were made.

| Protein | LexA | Bcl-2 | Bcl-xL | Bcl-xS | Al | Mcl-1 | Bad | Ced-9 | Bax | Bak |
|---------|------|-------|--------|--------|----|-------|-----|-------|-----|-----|
| Binding | +    | +     | −      | +      | −  | +     | +   | +     | +   | −   |
| Suppression | 1.00 | 5.18  | 5.55   | 1.01   | 3.60 | 8.05  | 0.82 | 1.10  | NA  | ND  |

1 C. Kurschner and J. I. Morgan, unpublished data.
2 The abbreviations used are: GI, growth index; ICE, interleukin-1β-converting enzyme; TM, transmembrane.
When coexpressed with Bax, the majority of these mutants failed to bind to Bax and did not suppress death (Fig. 2, A and B). However, two Bcl-xL mutants, XF14 and XF15, were active in suppression of Bax toxicity but either bound weakly or not at all in the two-hybrid assay (Fig. 2A).

To ensure that these data were not a product of the LexA sequences, unfused XF14 and XF15 were examined for their suppressive effects on native Bax toxicity and for coimmunoprecipitation with MYC-tagged Bax. Both unfused XF14 (GI, 8.7) and XF15 (GI, 8.8) were potent suppressors of Bax lethality (GI, 1.0). For coimmunoprecipitation, the proteins were in vitro translated in the presence of [35S]methionine, whereas a MYC epitope-tagged Bax (MYC-Bax) was translated in the absence of the radionuclide. After appropriate mixing and incubation, Bcl-xL was specifically immunoprecipitated with MYC-Bax but not the MYC epitope alone (Fig. 3). In contrast, neither XF14 nor XF15 was precipitated specifically with MYC-Bax and the anti-MYC monoclonal antibody (Fig. 3). XF15 showed no evidence of Bax binding. XF14 gave a relatively high background immunoprecipitation that was not augmented by the presence of MYC-Bax, indicating that it does not bind to Bax. This analysis confirms a prior report using these and similar constructs (28).

**Bcl-xL and Bcl-xL Mutants Do Not Alter Bax Expression**—To preclude the possibility that coexpression of Bcl-xL or the mutants either reduced the expression or promoted the degradation of Bax, steady-state protein levels were determined by
immunoblotting. Fig. 4A shows the immunoblot analysis of Bax and Bcl-xL proteins in yeast. Cells were cotransformed with constructs encoding LexA-Bax and one of unfused Bcl-xL mutants, XF14 or XF15. An ~45-kDa LexA-Bax fusion protein accumulated to maximal levels within 12 h after galactose induction. Expression of ~26-kDa Bcl-xL, ~20-kDa XF14, and ~21-kDa XF15 immunoreactive bands were also detected 12 h after galactose induction. None of the anti-death proteins altered the levels or time course of LexA-Bax expression (Fig. 4A). Additional Bax-reactive proteins of lower molecular mass were also induced with similar kinetics to LexA-Bax. The levels of these proteins were also unaffected by Bcl-xL expression (Fig. 4A). It is unclear whether these bands are proteolytic fragments of LexA-Bax or incomplete transcription/translation products. No such proteins were observed when unfused Bax was expressed with Bcl-xL in yeast (Fig. 4B), suggesting that the additional bands do not represent selective processing of Bax sequences.

Whereas expression of Bax is lethal, yeast transformed with the Bax plasmid did grow if cultured for longer periods (data not shown). This phenomenon is important in that it has implications for the use of this system as a suppressor screening assay. The growth effect could arise in a number of ways. First, a mutation in the vector could either inhibit expression of Bax or render it biologically inactive. Second, yeast may produce a mutation in the vector could either inhibit expression of Bax or lose a target of Bax. To test the presence of Bcl-xL, this selective pressure is absent, and high expressing yeast that result in a loss of Bax expression. In the presence of Bcl-xL, this selective pressure is absent, and high (normally lethal) levels of full-length Bax are expressed. This effect must be considered when using this suppressor assay to identify proteins that functionally interact with Bax/Bak.

Bax Toxicity May Not Be Mediated by ICE-like Proteases—Since Bax and Bcl-xL may function independently, the mechanisms that mediate Bax toxicity were investigated. Another study has established that Bax lethality in yeast is not associated with DNA laddering (21), although it may involve proteolysis. Indeed, proteases belonging to the interleukin-1β-converting enzyme (ICE) subfamily (recently termed caspases) have been implicated in cell death in phylogenetically disparate species (2). A search of the yeast genome for the consensus active site of ICE proteases (QACRG) yielded no hits, suggesting that these enzymes could not mediate death in yeast. To further examine this point, yeasts were cotransformed with Bax and CrmA, an inhibitor of ICE proteases that is derived from cowpox virus (25). CrmA did not rescue Bax toxicity and alone had no effect upon yeast growth (data not shown). Together, the data indicate that ICE-like proteases do not mediate Bax lethality in yeast.

Bcl-2 and Bcl-xL Have Distinct Structure-Activity Relationships in Yeast—Several studies have suggested that Bcl-2 and Bcl-xL have distinct properties (15, 17, 18). Therefore, the structure-activity relationships for Bax dimerization and suppression were determined for the two molecules in yeast. Two regions of Bcl-2 and Bcl-xL distinguished the biological properties of the two proteins. We confirm that the transmembrane (TM) domain of Bcl-2 is not essential for suppression of Bax toxicity in yeast and its elimination does not affect Bax binding (see mutant BF3 in Fig. 2B) (21). However, elimination of the TM domain in Bcl-xL leads to both the loss of Bax binding and suppressor activity (see mutant XF3 in Fig. 2A). As shown above, deletion of the putative loop region in Bcl-xL (mutants
XF14 and XF15) (11) results in a loss of Bax binding but retention of Bax suppressor activity (Fig. 2A). Whereas an equivalent mutation in Bcl-2 (BF6) retained suppressor activity, it still bound well to Bax (Fig. 2B). These data suggest that the loop region may be important for the interaction of Bcl-xL with Bax, whereas the equivalent domain in Bcl-2 is not. Together, the results indicate that whereas Bcl-2 and Bcl-xL, both, can suppress Bax toxicity in yeast, they may not do so in an identical manner.

**DISCUSSION**

As in vertebrate cell death models, members of the Bcl-2 family can be grouped into three functional classes in yeast. The first group comprises proteins, such as Bax and Bak, which are lethal per se. The second group includes Bcl-2, Bcl-xL, Mcl-1, and A1, which bind to and suppress Bax and Bak lethality. The third group bind to anti- but not pro-apoptotic members of the Bcl-2 family. These proteins, which include Bad, Bcl-xS, and the C. elegans Ced-9, are functionally inactive in terms of direct killing or death suppression. In vertebrates, proteins such as Bad and Bcl-xS are not considered to be lethal per se but rather are thought to potentiate killing by binding to anti-apoptotic members of the Bcl-2 family (29, 30). Thus, mammalian Bcl-2 family members have the same spectrum of biological activities in yeast as they do in vertebrate cells.

One unexpected result was that whereas Ced-9 did bind to anti-apoptotic members of the Bcl-2 family, it did not bind to Bax and did not suppress Bax/Bak killing. Although we can find no study that has used Ced-9 to rescue death in a vertebrate cell, it is the presumed homolog of Bcl-2 (8) and was expected to suppress killing. However, the properties of Ced-9 are more akin to those of Bcl-xS than they are to Bcl-2. It is conceivable that Ced-9 binds specifically to a C. elegans homolog of Bax, although such a gene has been identified. However, the possibility exists that family members such as Ced-9 and Bcl-xS might exert their functions through mechanisms other than intrafamily binding.

Genetic analysis in C. elegans has shown that Ced-9 acts via Ced-4, a protein of unknown function, and Ced-3, an ICE-like cysteine protease (4). It is possible that Ced-9, and by implication Bcl-xS and Bad, might act by binding to Ced-4 or related proteins in vertebrates. However, whereas many studies have implicated cysteine proteases in cell death in vertebrates (2), it is unlikely that ICE-like proteases mediate Bcl-2 family effects in yeast. First, Bax lethality is not inhibited by coexpression of the ICE protease inhibitor, CrmA. Second, no consensus sequence for the active site of ICE proteases has been found in the yeast genome. Therefore, Bax lethality may not involve the activation of ICE-like proteases in yeast. Indeed, Bax killing has been shown to be independent of this class of proteases in at least one vertebrate model (31).

Analysis of the structural requirements of Bcl-xL for suppression of Bax toxicity in yeast revealed that deletions at both the N and C termini eliminated biological activity. In one mutant, XF13, deletion of the last 22 amino acids, which included the TM domain, resulted in loss of suppressor activity. This result suggests that membrane targeting is essential for the protective effect of Bcl-xL in yeast. However, the equivalent domain in Bcl-2 is not required for rescue (19), indicating that these two related proteins may have divergent mechanisms of action. Such a notion is underscored by the distinct phenotypes of mice that lack functional bcl-2 or bcl-x alleles and by the unique protective effects of Bcl-xL in some cell death models (14, 15, 17, 18). Whereas the TM domain is thought to be necessary for the activity of Bcl-xL in vertebrate cells, the situation for Bcl-2 is controversial (12, 32, 33). However, the recent demonstration that Bcl-xL has structural similarities to the pore-forming subunit of diphtheria toxin suggests that its biological function may occur at or within cellular membranes (11). Therefore it is conceivable that there are proteins in yeast that can dock Bcl-2, but not Bcl-xL, to membranes thereby obviating the requirement for a TM domain. Bcl-2 and Bcl-xL can also be discriminated by their structure-activity relationship for binding to Bax. Mutants of Bcl-xL that had the putative loop domain deleted (XF14 and XF15) did not bind Bax, whereas an equivalent deletion in Bcl-2 (BF6) did bind in the two-hybrid assay. Since the loop regions of Bcl-2 and Bcl-xL are not conserved, these data may be an indication that these domains can selectively modify dimerization, although they may not be part of the dimerization interface.

Dimerization between Bcl-2 family members is considered central to their biological activity. Indeed, in one model it is the stoichiometry of various pro- to anti-apoptotic Bcl-2 family members that is supposed to determine cell fate (34). However, the view that dimerization is the sole determinant of activity has been questioned. For example, some Bcl-xL mutants that failed to associate with Bax still rescued 70–80% of Sindbis virus-induced cell death (16). However, it could be argued that Sindbis virus-triggered cell death may not be mediated by Bax. Indeed, there is evidence of Bax-independent cell death pathways in bax-null mice (35). Recently, some Bcl-xL mutations were described that had reduced, or no, binding activity to Bax but that rescued vertebrate cells from death triggered by IL-3 deprivation (11, 28). Therefore we compared the activity of these deletions with other Bcl-xL and Bcl-2 mutants as suppressors of Bax toxicity in yeast. All full-length Bcl-2 family members and mutants that bound to Bax inhibited killing, whereas those that did not bind were inactive. However, the two internal deletion mutants of Bcl-xL, XF14 and XF15, that did not bind to Bax were potent suppressors. This indicates that dimerization is not essential for Bcl-xL to suppress Bax lethality in yeast.

The observation that Bcl-xL can antagonize Bax killing in yeast independent of heterodimerization has several important implications. First, it argues against the effects of Bax in yeast as being nonspecific toxicity that can be attenuated by any Bax-binding protein. Second, it suggests that Bax and Bcl-xL either interact with, or compete for, a common downstream target or pathway in yeast. Alternatively, they could influence antagonistic mechanisms. Third, the data suggest that Bax and Bcl-xL act upon mechanisms that have been conserved from yeast to mammals, although these processes may only have been adapted for the control of cell elimination in multicellular organisms. The assay described here can be used as a genetic suppressor screen to identify potential components of this pathway in yeast and vertebrates.

**Acknowledgments**—We thank Dr. Craig B. Thompson for helpful discussions and Dr. Steven Dalton for providing the yeast expression plasmids pSD.10a and Yعقب lex, in vitro translation plasmids, and the S. cerevisiae reporter strain S260.

**REFERENCES**

1. Ellis, R. E., Yuan, J., and Horvitz, H. R. (1991) *Annu. Rev. Cell Biol.* 7, 663–698
2. Hale, A. J., Smith, C. A., Sutherland, L. C., Stoneman, V. E. A., Longthorne, V. L., Culhane, A. C., and Williams, G. T. (1996) *Eur. J. Biochem.* 236, 1–26
3. Miura, M., and Yuan, J. (1996) *Curr. Top. Dev. Biol.* 32, 139–174
4. Hengartner, M., Ellis, R., and Horvitz, H. (1992) *Nature* 356, 494–499
5. Tsujimoto, Y., Cosman, J., Jaffe, E., and Croce, C. (1985) *Science* 228, 1440–1443
6. Tsujimoto, Y., and Croce, C. R. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 5214–5218
7. Reed, J. (1994) *J. Cell Biol.* 124, 1–6
8. Hengartner, M. O., and Horvitz, H. R. (1994) *Cell* 76, 665–676
9. Williams, G. T., and Smith, C. A. (1990) *Cell* 74, 777–779
10. Zha, H., Aime-Sempe, C., Sato, T., and Reed, J. C. (1996) *J. Biol. Chem.* 271, 7440–7444
11. Muchmore, S. M., Sattler, M., Liang, H., Meadows, R. P., Harlan, J. E., Yoon, H. P., Nethsetshe, D., Chang, B. S., Thompson, C. B., Wong, S.-L., Ng, S.-C.,...
and Fesik, S. W. (1996) *Nature* **381**, 335–341
12. Oltvai, Z. N., Milliman, C. L., and Korsmeyer, S. J. (1993) *Cell* **74**, 609–619
13. Sato, T., Hanada, M., Bodrug, S., Irie, S., Iwama, N., Boise, L. H., Thompson, C. B., Golemis, E., Fong, L., Wang, H.-G., and Reed, J. C. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 9238–9242
14. Nakayama, K.-i., Nakayama, K., Negishi, I., Kuida, K., Shinkai, Y., Louie, M. C., Fields, L. E., Lucas, P. J., Stewart, V., Alt, F. W., and Loh, D. Y. (1995) *Science* **261**, 1584–1588
15. Motoyama, N., Wang, F., Roth, K. A., Sawa, H., Nakayama, K.-i., Nakayama, K., Negishi, I., Senju, S., Zhang, Q., Fuji, S., and Loh, D. Y. (1995) *Science* **267**, 1506–1509
16. Cheng, E. H.-Y., Levine, B., Boise, L. H., Thompson, C. B., and Hardwick, J. M. (1996) *Nature* **379**, 554–556
17. Tuscano, J. M., Druey, K. M., Riva, A., Pena, J., Thompson, C. B., and Kehrl, J. H. (1996) *Blood* **88**, 1359–1364
18. Gottschalk, A. R., Boise, L. H., Thompson, C. B., and Quintans, J. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 7350–7354
19. Hanada, M., Aimé-Sempé, C., Sato, T., and Reed, J. C. (1995) *J. Biol. Chem.* **270**, 11962–11969
20. Greerhal, W., Stephan, C., and Chaudhuri, B. (1996) *FEBS Lett.* **380**, 169–175
21. Zha, H., Fisk, H. A., Yaffe, M. P., Mahajan, N., Herman, B., and Reed, J. C. (1996) *Mol. Cell. Biol.* **16**, 6494–6508
22. Kurschner, C., and Morgan, J. I. (1995) *Mol. Brain Res.* **37**, 249–258
23. Kurschner, C., and Morgan, J. I. (1995) *Mol. Cell. Biol.* **15**, 246–254
24. Dalton, S., and Treisman, R. (1992) *Cell* **68**, 597–612
25. Ray, C., Black, R., Kronheim, S., Greenstreet, T., Sleigh, P., Salvesen, G., and Pickup, D. (1992) *Cell* **68**, 597–604
26. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) in *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
27. Gallagher, S., and Hurrel, J. G. R. (1993) in *Current Protocols in Molecular Biology*, pp. 10.8.1–10.8.14, John Wiley & Sons, Inc., NY
28. Chang, B. S., Minn, A. J., Muchmore, S. W., Fesik, S. W., and Thompson, C. B. (1997) *EMBO J.* **16**, 968–977
29. Yang, E., Zha, J., Keck, J., Boise, L. H., Thompson, C. B., and Korsmeyer, S. J. (1995) *Cell* **80**, 285–291
30. Boise, L. H., Gonzalez-Garcia, M., Postema, C. E., Ding, L., Lindsten, T., Turk, L. A., Mao, X., Nunez, G., and Thompson, C. B. (1993) *Cell* **74**, 597–608
31. Xiang, J., Chao, D. T., and Korsmeyer, S. J. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 14559–14563
32. Borner, C., Martinou, I., Mattmann, C., Irmler, M., Schaere, E., and Martinou, J.-C. (1994) *J. Cell Biol.* **126**, 1059–1068
33. Hunter, J. J., Bond, B. L., and Parslow, T. G. (1996) *Mol. Cell. Biol.* **16**, 877–883
34. Oltvai, Z. N., and Korsmeyer, S. J. (1994) *Cell* **79**, 189–192
35. Knudson, C. M., Tung, K. S. K., Tourtellotte, W. G., Brown, G. A. J., and Korsmeyer, S. J. (1995) *Science* **270**, 96–98