The BCL-2 proto-oncogene contains unusually long untranslated 5′ and 3′ sequences. Deletion of the sequences flanking the BCL-2 open reading frame dramatically increases the level of protein expression. Transient high level BCL-2 protein expression mediated by plasmid transfection or by infection with recombinant adenovirus results in potent apoptosis of several cell lines. Detailed mutational (deletion and add-back) analysis reveals that both 5′- and 3′-flanking sequences contribute to the negative modulation of protein expression from the BCL-2 open reading frame. It appears that these sequences exert the negative regulatory effect in an orientation-dependent manner. Analysis of BCL-2 RNA levels indicate that elevated levels of mRNA may be the primary cause of elevated levels of protein expression. Apoptosis induced by adenovirus vectors expressing elevated levels of BCL-2 can be readily inhibited by the caspase inhibitor z-vAD-fmk, suggesting that high levels of BCL-2 expression induce apoptosis via the caspase cascade. Mutational analysis of BCL-2 indicates that its pro-apoptotic activity is separable from its anti-apoptosis activity. Our results raise the possibility that oncogenic conversion of BCL-2 may require somatic mutations in the pro-apoptotic activity, in addition to other activating mutations that result in enhanced expression. Consistent with this hypothesis, a somatic mutation of BCL-2 observed in multiple human tumors results in reduced apoptosis activity.

It is well established that the BCL-2 proto-oncogene suppresses apoptosis induced by a multitude of stimuli in a variety of cell types (1, 2). In a seminal study by Vaux et al. (3), BCL-2 was shown to extend the survival of growth factor-dependent hematopoietic progenitor cells after factor withdrawal. In subsequent studies by Hockenbery et al. (4), it was shown that BCL-2 promotes survival of such factor-dependent cells by suppressing apoptosis. Most studies on the anti-apoptosis activity of BCL-2 have been based on stable cell lines (selected after transfection of BCL-2 expression vectors) that ectopically express elevated levels of BCL-2. In addition to the anti-apoptosis activity, recent studies have revealed that BCL-2 exhibits other novel activities. For example, BCL-2 has been shown to inhibit cell proliferation (5–9), which is a function separable from the anti-apoptosis activity (6, 9). BCL-2 has also been shown to promote cell proliferation in the central nervous system (10).

The human BCL-2 gene is characterized by unusually long 5′- and 3′-untranslated regions (11–13), including the entire first exon (14). The functions of the unusually long untranslated regions are not known. Interestingly, the 5′-untranslated region contains a sequence element that negatively regulates translation of BCL-2 (15). Here, we show that deletion of the 5′- and 3′-flanking sequences results in a large increase in BCL-2 protein expression, which results in cell death via the CED-3/ICE family caspase cascade.

**Experimental Procedures**

**Plasmids**—pcDNA3Bcl2 was constructed by cloning an EcoRI fragment from the plasmid pSFFV-BCL-2 (no. 58) (14) into pcDNA3 cut with EcoRI, pcDNA3-Bcl2UTR (no. 58) was prepared by cloning a PCR-generated DNA fragment (digested with HindIII and SstI) and cloned between the HindIII and XhoI sites of pcDNA3. The PCR product was generated using oligonucleotides, 5′-GATCACGCTTTCCATGGCGCAGCTGGGAGA-3′ (primer 1) and 5′-ACCGGCTGCACTACTTGGTGGCCAGATAG-3′ (primer 2) as primers and the BCL-2 cDNA clone no. 58 (14) as the template. pcDNA3-Bcl2UTR (B4) was constructed similarly using pB4 (11) as the template. pcDNA3-mBcl2 constructed by cloning the EcoRI/XhoI fragment containing the mouse BCL-2 ORF from pUC19 (16) into pcDNA3. pcDNA3-Bcl2UTR + 5′-UTR was prepared by cloning a DNA fragment generated using the primer 5′-GTAGGGGTCCCTGGGAGAGGTGCCGTTGGC-3′ (primer 3) and primer 2. The PCR product was digested with SstI and cloned into the EcoRV and XhoI sites of pcDNA3. pcDNA3-Bcl2UTR + 3′-UTR was made by replacing a C-terminal fragment (BamHI/XhoI) of pcDNA3-Bcl2UTR with a C-terminal fragment (BamHI/XhoI) from pcDNA3-Bel2. pcDNA3-Bcl2UTR + 3′-UTR was constructed by replacing a C-terminal fragment (SacII/XhoI) of pcDNA3-Bcl2UTR + 5′-UTR with that of pcDNA3-Bcl2UTR + 3′-UTR. pcDNA3-Bcl2 + 5′-UTR was generated by digestion of pcDNA3-Bcl2 with HindIII and EcoNI (partial) followed by blunt-end ligation. pcDNA3-Bcl2 + 3′-UTR was made by cloning the fragment generated by PsI (blunted) and EcoRI from pSFFV-Bcl2 into pcDNA3 between EcoRI and EcoRV sites. pcDNA3-Bel2 + 5′-UTR was made by deletion of a KpnI and EcoNI (partial) fragment from pcDNA3-Bel2 + 3′-UTR. pcDNA3-Bcl2 + 3′-UTR-I was constructed by reversing the orientation of the appropriate PsI-generated fragment of pcDNA3-Bel2 (no. 58). pcDNA3-Bcl2 + 3′-UTR-H by substituting a 1-kb fragment of pBR322 (NheI and blunted BsmI) for a 1-kb fragment of pcDNA3-Bel2 (no. 58) between PstI (partial, blunt end) and XhoI sites. All PCR reactions were carried out using Vent DNA polymerase (New England Biolabs) in 5% MgSO. The clones derived from PCR amplification were sequenced by dyeoxy or by automated sequencing.

**Adenovirus Expression Vectors**—The BCL-2 expression cassettes, from plasmids pcDNA3-Bcl2 and pcDNA3-Bcl2UTR, and the lacZ expression cassette, from plasmid pCMV-β-galactosidase, were cloned into the adenovirus transfer vector pAdLTV (17) between unique XhoI...
and BgII sites. To construct the recombinant adenoviruses, 5 μg of each of the transfer plasmid was cotransfected with 5 μg of the adenovirus genomic plasmid pBHG33 (18) into 293 cells by the calcium phosphate method. Seven days after transfection, plaques were picked and screened for the presence of the transgene by restriction analysis. Positive plaques were further purified through a second plaque assay, amplified and titrated in 293 cells. In these recombinants, the transgenes are expressed under the control of CMV-IE promoter and interleukin-2 poly(A) site.

Cell Death Assays—Transient cell death assays were carried out using the human 293 cell line. Cells were transfected at 60% confluency with 1.5 μg of various expression plasmids and 0.4 μg of the reporter plasmid pCMV-β-galactosidase by the calcium phosphate method in 12-well plates. Eight hours after transfection cells were fixed and stained with X-gal (19). The number of blue cells was counted, and the number of round (apoptotic) cells compared with the total blue cell count was determined.

Two different cell death assays were used to determine the effect of adenovirus vectors. In the first assay, cell viability was determined by vital stain exclusion (trypan blue). In the second assay, cell viability was determined by measuring mitochondrial viability (MTT assay). In the vital dye exclusion assay, 5 × 10⁶ cells/35-mm dish were infected with 100 plaque-forming units of various viruses in 2 ml of DMEM containing 10% fetal calf serum. Twenty-four hours after infection, adherent cells were treated by treatment with 0.5 ml of trypsin-EDTA solution and combined with the floating cells collected from the culture media. The suspension was mixed with an equal volume of trypan blue (0.4% solution in phosphate-buffered saline). Cells that excluded the dye were counted. For MTT assays (20), cells were plated in 96-well tissue culture plates in DMEM containing 10% fetal calf serum and incubated overnight. The following day, triplicate wells of cells at approximately 50% confluency were infected with various multiplicities of infection resulting in an infection rate of ≥90% (as determined by X-gal staining of companion cultures infected with Ad-lacZ). Viruses were added in 50 μl of DMEM containing 2.5% fetal bovine serum for 4 h at 37 °C. An additional 150 μl of DMEM containing 2.5% fetal bovine serum were then added, and the cells were cultured overnight. After an overnight infection, the media were replaced with 200 μl of DMEM containing 10% fetal calf serum. At 45–48 h post-infection, 50 μl of MTT (Sigma M-5655, 5 mg/ml stock in phosphate-buffered saline, diluted to 1 mg/ml of culture medium) were added and incubated for additional 4 h. Media were aspirated, cell monolayers were solubilized in 150 μl of Me₂SO, and the absorbance at 545 nm was determined. The data are plotted as percentages of the average of 12 wells of noninfected cells used as the control.

Analysis of DNA Fragmentation—293 cells (1 × 10⁶ cells/60-mm dish) were infected at 100 plaque-forming units/cell. Twenty-four hours later, adherent and floating cells were collected and lysed, and small molecular weight DNA was prepared by Hirt extraction (21), treated with RNase, and analyzed on a 1% agarose gel.

Western Blot Analysis—3.4 × 10⁵ MCF-7 cells were plated in 24-well plates containing 10% fetal calf serum. Twenty-four hours after plating, cells were infected at 300 plaque-forming units/cell in 250 μl of serum-free DMEM for 4 h at 37 °C. Virus-containing medium were aspirated, and replaced with fresh DMEM containing 2.5% fetal calf serum. At various times post-infection, adherent cells from triplicate wells were harvested and pooled, and lysed in SDS-polyacrylamide gel electrophoresis sample buffer, and the equivalent of 7.5 × 10⁵ adherent cells were loaded per lane of a 16% minigel (Novex). Following SDS-polyacrylamide gel electrophoresis, the proteins were electrotransferred onto a 0.2-μm nitrocellulose membrane and probed with a mouse anti-human BCL-2 primary antibody (Dako, MO887) at 1:300 dilution, followed by a sheep anti-mouse-horseradish peroxidase secondary antibody (Amersham NAB31 used at 1:5000 dilution) and chemiluminescent detection (Amersham Corp.). For the analysis of transiently transfected cells, 293 cells were transfected at 60% confluency in 35-mm dishes with 4 μg of total DNA. Nine and a half hours later, cells were lysed in 100 μl of electrophoresis sample buffer, and 10-μl samples were loaded onto a 15% gel SDS-polyacrylamide gel electrophoresis, followed by transfer to a nitrocellulose membrane. A rabbit anti-glutathione S-transferase-BCL-2 polyclonal serum (1:1000) and donkey anti-rabbit-horseradish peroxidase antibodies (Amersham NAB944 at 1:3000) were used to detect BCL-2.

Northern Blot Analysis—Total cytoplasmic RNA was isolated using RNAzol B solution (TEL-TEST Inc.) and purified by CsCl density gradient centrifugation. 12 μg of RNA were loaded in each lane, subjected to electrophoresis in a formaldehyde-agarose gel, transferred to a Duralon nylon membrane (Stratagene), and hybridized with the BCL-2 probe. The probe was prepared by random primed extension (DuPont) and the HindIII/XbaI fragment from pcDNA3-Bcl2A2UTR as the template.

RESULTS

Transient Expression of BCL-2—The human BCL-2 cDNA contains 1.4 kb of 5'- and 3.9 kb of 3'-untranslated sequences (Fig. 1A). A widely used cDNA clone (clone no. 58) (14) contains 58 bp of 5'- and 900 bp of 3'-untranslated sequences. We constructed a derivative of this cDNA clone by PCR that lacks the untranslated sequences (pcDNA3-Bcl2A2UTR). We observed that, after transfection of pcDNA3-Bcl2ΔUTR, the number of G418-resistant colonies induced was substantially lower than that induced either by the pcDNA3 empty vector or the vector expressing the parental cDNA (pcDNA3-Bcl2). This finding prompted us to examine whether the clone lacking the untranslated regions (pcDNA3-Bcl2ΔUTR) caused any cell death in transfected cells. For this purpose, 293 cells were transfected with a reporter plasmid expressing the Escherichia coli lacZ gene along with the pDNA3 vector or pcDNA3-Bcl2 or pcDNA3-Bcl2ΔUTR. Cells expressing β-galactosidase were detected by X-gal staining, and the percent of apoptotic cells was calculated from the number of round and fragmenting blue cells out of the total number of blue cells (19). Transfection of the pcDNA3 vector or the vector expressing the parental cDNA (pcDNA3-Bcl2) did not induce significant cell death (Fig. 1B). In contrast, about 80% of the cells transfected with pcDNA3-Bcl2ΔUTR were round. The observed cell death activity of BCL-2 was confirmed with two different independently derived clones that lack the flanking sequences (not shown). None of the pcDNA3-Bcl2ΔUTR clones contained any mutations compared with parental cDNA. Similar results were also obtained with a different human BCL-2 cDNA (11) and a mouse BCL-2 cDNA (22).

Adenovirus Vector-mediated Expression of BCL-2—To further substantiate the above results, we constructed recombinant adenoviruses that express the two versions of the BCL-2 cDNA. The effects of the recombinant adenoviruses on cell viability of three different human cell lines were determined by the MTT assay. Infection of cell lines MRC-5, MCF-7, and DU145 with an adenovirus vector expressing the E. coli lacZ gene (AdΔE1-lacZ) did not result in significant cell death compared with uninfected control cells (Fig. 2). Similarly, infection with the vector that expresses the parental cDNA clone (AdΔE1-Bcl2) also did not result in any significant cell death. In contrast, infection with the adenovirus vector expressing the BCL-2 cDNA lacking the flanking sequences (AdΔE1-Bcl2ΔUTR) resulted in severe cell death in MRC-5 (Fig. 2A). Similarly, infection of MCF-7 and DU145 cells with AdΔE1-Bcl2ΔUTR also resulted in significant cell death (Fig. 2, B and C), although the effect on DU145 was less pronounced (Fig. 2C). These results indicate that the BCL-2 cDNA lacking the 5'- and 3'-flanking sequences induce cell death during transient expression. It should be noted that the DU145 cell line is resistant to apoptosis induced by Fas and tumor necrosis factor-α (22).

BCL-2 mRNA and Protein Expression—To determine the possible mechanism by which the flanking sequences dictate the opposing activities of BCL-2, we determined the levels of expression of BCL-2 mRNA and protein. These levels were determined from MCF-7 cells infected with AdΔE1-Bcl2ΔUTR or AdΔE1-Bcl2. Total cytoplasmic RNA was extracted from infected cells (adherent and floating) and analyzed by Northern blot analysis. In cells infected with AdΔE1-Bcl2, an mRNA band of 2.6 kb (that corresponds to the BCL-2 ORF and flanking vector sequences) was observed (Fig. 3A). In cells infected with AdΔE1-Bcl2ΔUTR, significantly higher levels of a 1.2-kb
mRNA were observed. These results suggest that deletion of the flanking sequences of the BCL-2 cDNA significantly increases the level of BCL-2 mRNA expression.

The levels of BCL-2 protein expression in cells infected with the adenovirus vectors expressing the two versions of the BCL-2 cDNA were also analyzed (Fig. 3B). Adherent and floating cells were collected at various times after infection and analyzed by Western blot analysis. At various times after infection, the level of BCL-2 protein expression was consistently higher in cells (both adherent and floating) infected with AdΔE1-Bcl2ΔUTR. Similar results were also obtained with two other cell lines infected with adenovirus vectors and in cells transiently transfected with plasmid expression vectors (results not shown). These results clearly indicate that deletion of the flanking sequences of the BCL-2 cDNA dramatically increases the level of mRNA and protein expression.

**Mutational Analysis of the Flanking Sequences**—To determine the role of the 5' and 3'-flanking sequences separately, we constructed a series of add-back mutants by adding either the 5' or the 3'-flanking sequences (by PCR) to pcDNA3-Bcl2ΔUTR (Fig. 4A). Similarly, a series of 5'- or 3'-deletion mutants of pcDNA3-Bcl2 were also constructed by exploiting conveniently located restriction sites (Fig. 4B). The cell death activities of the various mutants were then determined by transient transfection (Fig. 4D) as described in the legend to Fig. 1B. Addition of the 5'-flanking sequences to pcDNA3-Bcl2ΔUTR (+5' in Fig. 4D) reduced the cell death activity of pcDNA3-Bcl2ΔUTR. Similarly, addition of the 3'-flanking sequences also reduced cell death activity (+3' in Fig. 4D). Addition of both 5'- and 3'-flanking sequences significantly reduced the cell death activity, close to the level of pcDNA3-Bcl2. Comparable results were obtained by analysis of deletion mutants (Fig. 4B). Deletion of the 5'-untranslated region (−7; EcoNI) increased the apoptosis activity of pcDNA3-Bcl2. Similarly, deletion of the 3'-flanking sequences (+752; PstI) also increased the apoptotic activity. Deletion of both 5'- and 3'-flanking sequences increased the apoptotic activity in an additive manner. These results provide evidence that both 5'- and 3'-flanking sequences have a combinatorial effect in modulating the cell death activity of BCL-2.

To determine if the flanking sequences have any orientation-independent effect, we examined the effect of a construct in which the orientation of the 3'-flanking sequence was inverted (+3'-UTR-I in Fig. 4C). Similarly, we also examined the effect of a construct in which we substituted the 3'-flanking sequence with a heterologous DNA (+3'-UTR-H in Fig. 4C). The apoptotic activities of both these constructs were similar to that of the construct that lacks the 3'-UTR (Δ3'-UTR), suggesting that the flanking sequences may function only in transcriptional orientation (Fig. 4E).
Effect of BCL-2 Mutations—To determine if the apoptotic activity of BCL-2 is linked to its anti-apoptotic activity, we examined the effect of two previously characterized mutants, G145E (BH1) and W188A (BH2) which were found to be severely defective in the anti-apoptotic activity (24). The BH2 mutant (W188A) did not significantly affect the apoptotic activity (Fig. 5). In contrast, the BH1 mutant (G145E) exhibited significantly reduced apoptotic activity (Fig. 5). These results suggest that the pro- and anti-apoptotic activities of BCL-2 are separable and that a protein region located around the BH1 domain may be important for the apoptotic activity.

Effect of Caspase Inhibitor—Since CED-3/ICE family caspases are activated during apoptosis induced by several different stimuli (reviewed in Refs. 25 and 26), we determined if the cell death induced by higher levels of BCL-2 expression is also dependent on caspases (Fig. 6, A and B). 293 cells infected with AdΔE1-BCL-2ΔUTR induced extensive internucleosomal DNA fragmentation (Fig. 6, left panel). In contrast, there was no significant DNA fragmentation in mock-infected cells or in cells infected either with AdΔE1-lacz or AdΔE1-BCL-2. We then examined if the caspase inhibitor z-VAD-fmk can inhibit DNA fragmentation induced by AdΔE1-BCL-2ΔUTR. Addition of 25 or 50 μM z-VAD-fmk significantly inhibited the extent of DNA fragmentation induced by AdΔE1-BCL-2ΔUTR. Similarly, addition of z-VAD-fmk also inhibited the extent of cell death (determined by vital dye exclusion) induced by AdΔE1-BCL-2ΔUTR (Fig. 6B). These results suggest that a distal step in the cell death pathway activated by infection with AdΔE1-Bcl2ΔUTR may involve caspases.

Effect of Oncogenic Mutant—Expression of BCL-2 is activated in a number of human tumors. Our results raise the possibility that oncogenic conversion of BCL-2 may require somatic mutations in the pro-apoptotic activity, in addition to other activating mutations that result in enhanced expression. To test this hypothesis, we examined the apoptotic activity of a BCL-2 mutant, P59S, located within the nonconserved region of BCL-2. This mutant was chosen since mutations involving the Pro59 residue (Pro59→Ser or Pro59→Leu) have been detected in three different human non-Hodgkin’s lymphomas (27, 28). The P59S mutant has previously been shown to efficiently protect against apoptosis (27). Transient transfection of the plasmid containing this mutant (pcDNA3-Bcl2UTR/P59S; lacking the flanking sequences) or pcDNA3 Bcl2ΔUTR expressed elevated levels of BCL-2 compared with cells transfected with pcDNA3-Bcl2 (wild type) (Fig. 7A), as expected. Transfection of pcDNA3-Bcl2ΔUTR/P59S consistently exhibited less pronounced cell death compared with pcDNA3-Bcl2ΔUTR (Fig. 7B). These results suggest that at least one of the somatic mutations observed in human tumors exhibit a reduced apoptosis activity.

DISCUSSION

In this communication, we have identified a surprising cell death activity of BCL-2 manifested during high levels of transient protein expression. We believe that a similar activity has not thus far been identified since most assays used to determine the activity of BCL-2 have employed cell lines that have been selected to grow in tissue culture. It should be noted that cell lines established by transfection of pcDNA3-Bcl2ΔUTR also protect against cell death similar to cell lines established by transfection of pcDNA3-Bcl2 (not shown), suggesting that

---

2 E. J. Uhlmann and G. Chinnadurai, unpublished results.
cells transfected with pcDNA3-Bcl2ΔUTR get selected for survival during the selection procedure. We believe that our results may, at least partially, explain the effect of BCL-2 reported by Pietenpol et al. (5). These investigators have observed that transfection of a BCL-2 expression vector in certain tumor cell lines strongly inhibited G418-resistant colony formation.

Employing one of the tumor cell lines, SW480, used by these investigators, we have observed that transfection of pcDNA3-Bcl2 (not shown).

Our study does not address the physiological relevance of the

FIG. 4. Mutational analysis of the untranslated regions. 5'- and 3'-untranslated sequences were added to the ΔUTR construct (panel A) or removed from the cDNA clone no. 58 (panel B). In panel C, the 3'-untranslated sequences were inverted (+3'-UTR-I) or substituted with heterologous sequence (+3'-UTR-H). Transient cell death assays with various mutants were carried out in 293 cells and given in panel D (for mutants shown in panels A and B) and in panel E (for mutants shown in panel C).
cell death activity of BCL-2. However, the observation that CED-9, the Caenorhabditis elegans anti-cell death protein also contains a minor cell death activity (29) raises the possibility that the cell death activity of BCL-2 may also be physiologically relevant under certain yet unidentified settings. It could be speculated that the unusually long flanking sequences of the BCL-2 gene might have evolved to regulate the opposing activities of BCL-2 protein. Under certain physiological or pathological conditions, shorter transcripts may be generated, and such transcripts may be preferentially translated leading to cell death rather than survival. There is some basis for such a speculation, because the BCL-2 gene has also been shown to be transcribed from a minor promoter (30), resulting in a transcript that would have a 5’ end similar to the 5’ ends of the mutants described here. The presence of a separate cis-acting translational inhibitory sequence in the 5’-untranslated sequences (15) also lends support for negative regulation of BCL-2 protein expression by flanking sequences. The modulation of BCL-2 expression by the flanking sequences would be similar to the role of the 3’-flanking sequences in regulation of p53 protein expression in response to DNA damage (31).

In the present study, we have observed that deletion of the UTRs enhanced BCL-2 mRNA accumulation and protein expression. It is possible that transcriptional and post-transcriptional regulation such as mRNA stability may contribute to increased accumulation of BCL-2 mRNA. Although we have observed both elevated levels of mRNA and protein expression, the role of flanking sequences in negative modulation of translational efficiency cannot be ruled out. Interestingly, we have observed that both 5’- and 3’-flanking sequences of the BCL-2 mRNA could potentially form extensive secondary structures (not shown). It is possible that such secondary structures may also suppress translational efficiency of the BCL-2 mRNA.

The molecular basis by which elevated levels of BCL-2 induce cell death is not known. It is possible that, during high level expression of BCL-2, N-terminal-deleted versions are expressed from internal translational initiation sites. Under certain conditions, we have observed a 24-kDa polypeptide (see Fig. 3B), in addition to the 26-kDa polypeptide. We believe that the 24-kDa polypeptide is translated from an internal initiation codon corresponding to Met16 since a BCL-2 mutant ΔUTR/M16L does not produce the 24-kDa band (not shown). The mutant M16L (Met16→Leu) still induces cell death, suggesting that the cell death activity observed in our studies may be primarily contributed by the 26-kDa BCL-2. However, we cannot fully rule out the role of other truncated versions generated during high levels of expression being pro-apoptotic. A hypothesis would be that higher levels of BCL-2 might result in formation of dimers and oligomers that may be pro-apoptotic. Under conditions of relatively lower levels of protein expression, BCL-2 may function as a monomer with anti-apoptosis activity. In this context, it has been shown that BCL-xL folds as a monomer in solution (32). Recent studies indicate that both BCL-xL (32) and BCL-2 form membrane pores (33, 34), and BCL-2 has been shown to block leakage of cytochrome c into the cytosol (35, 36). BCL-2 has been shown to form channels with larger conductances, suggesting the formation of BCL-2 dimers and oligomers in the membrane (33). It is possible that, above a threshold level, BCL-2 may cause constitutive loss of cytochrome c or other apoptotic factors from the mitochondria by forming megapores. BCL-2 retains the BH3 domain essential for the death activity of the pro-apoptotic proteins of the BCL-2 family (37–39). Our mutational analysis suggests that pro-
mutations expressed at high levels that we have examined
function mutants that contribute to enhanced oncogenesis.

apoptotic and anti-apoptotic activities of BCL-2 are separable. It
remains to be determined if the cell death activity of BCL-2 is
mechanistically similar to the known pro-apoptotic proteins.
Recent studies from several laboratories suggest that CED-9
promotes cell survival by simultaneously sequestering the cell
death proteins CED-4 and CED-3 (reviewed in Refs. 40–42). If
BCL-2 also functions by a similar mechanism, it is possible that
homodimerization of BCL-2 (at higher levels of expression)
may result in unsequestered BCL-2 interacting pro-apoptotic
proteins resulting in cell death.

The two opposing activities of the BCL-2 gene appear to be
similar to the activities of other oncogenes, such c-myc and Ela,
which promote cell proliferation as well as cell death.
Interestingly, two pro-apoptotic proteins of the BCL-2 family,
BAK (43) and BAX (44) have been reported to suppress apoptosis under
similar to the activities of other oncogenes, such c-myc and Ela,
which promote cell proliferation as well as cell death.
Interestingly, two pro-apoptotic proteins of the BCL-2 family,
BAK (43) and BAX (44) have been reported to suppress apoptosis under
similar to the activities of other oncogenes, such c-myc and Ela,
which promote cell proliferation as well as cell death.
Interestingly, two pro-apoptotic proteins of the BCL-2 family,
BAK (43) and BAX (44) have been reported to suppress apoptosis under
similar to the activities of other oncogenes, such c-myc and Ela,
which promote cell proliferation as well as cell death.
Interestingly, two pro-apoptotic proteins of the BCL-2 family,
BAK (43) and BAX (44) have been reported to suppress apoptosis under
similar to the activities of other oncogenes, such c-myc and Ela,
which promote cell proliferation as well as cell death.
Interestingly, two pro-apoptotic proteins of the BCL-2 family,
BAK (43) and BAX (44) have been reported to suppress apoptosis under
similar to the activities of other oncogenes, such c-myc and Ela,
which promote cell proliferation as well as cell death.
Interestingly, two pro-apoptotic proteins of the BCL-2 family,
BAK (43) and BAX (44) have been reported to suppress apoptosis under
similar to the activities of other oncogenes, such c-myc and Ela,
which promote cell proliferation as well as cell death.
Interestingly, two pro-apoptotic proteins of the BCL-2 family,
BAK (43) and BAX (44) have been reported to suppress apoptosis under
similar to the activities of other oncogenes, such c-myc and Ela,
which promote cell proliferation as well as cell death.
Interestingly, two pro-apoptotic proteins of the BCL-2 family,
BAK (43) and BAX (44) have been reported to suppress apoptosis under
similar to the activities of other oncogenes, such c-myc and Ela,
which promote cell proliferation as well as cell death.
Interestingly, two pro-apoptotic proteins of the BCL-2 family,
BAK (43) and BAX (44) have been reported to suppress apoptosis under
similar to the activities of other oncogenes, such c-myc and Ela,
which promote cell proliferation as well as cell death.
Interestingly, two pro-apoptotic proteins of the BCL-2 family,
BAK (43) and BAX (44) have been reported to suppress apoptosis under
similar to the activities of other oncogenes, such c-myc and Ela,
which promote cell proliferation as well as cell death.
Interestingly, two pro-apoptotic proteins of the BCL-2 family,
BAK (43) and BAX (44) have been reported to suppress apoptosis under
similar to the activities of other oncogenes, such c-myc and Ela,
which promote cell proliferation as well as cell death.
Interestingly, two pro-apoptotic proteins of the BCL-2 family,
BAK (43) and BAX (44) have been reported to suppress apoptosis under
similar to the activities of other oncogenes, such c-myc and Ela,