Molecular Cloning and Characterization of Bif-1
A NOVEL Src HOMOLOGY 3 DOMAIN-CONTAINING PROTEIN THAT ASSOCIATES WITH Bax*

Received for publication, February 19, 2001, and in revised form, March 12, 2001
Published, JBC Papers in Press, March 20, 2001, DOI 10.1074/jbc.M101527200

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Bax is a proapoptotic member of the Bcl-2 protein family that commits the cell to undergo programmed cell death in response to apoptotic stimuli. To gain further insights into Bax mechanisms, we have identified a novel Bax-binding protein, termed Bif-1, by using a yeast two-hybrid cloning technique. Bif-1 is an evolutionarily conserved cytoplasmic protein that contains a predicted Src homology 3 (SH3) domain located near its C terminus but shares no significant homology with members of the Bcl-2 family. A Northern blot analysis indicates that Bif-1 is expressed in most tissues with abundant expression in heart and skeletal muscle. Bif-1 is capable of interacting with Bax as demonstrated by yeast two-hybrid, coimmunoprecipitation, and immunofluorescence studies. Induction of apoptosis in murine pre-B hematopoietic cells FL5.12 by interleukin-3 withdrawal results in increased association of Bax with Bif-1, which is accompanied by a conformational change in the Bax protein. Overexpression of Bif-1 promotes Bax conformational change, caspase activation, and apoptotic cell death in FL5.12 cells following interleukin-3 deprivation. Bif-1 thus represents a new type of regulator of Bax-mediated signaling pathways for apoptosis.

Programmed cell death, or apoptosis, is defined as a physiological process that plays a critical role in the normal development and maintenance of tissue homeostasis by eliminating infected, mutated, or damaged cells in essentially all multicellular organisms (1, 2). Dysregulation of this physiological cell death process, resulting in defects in normal cell turnover, is implicated in the pathogenesis of many types of diseases, including cancer, autoimmune disease, neurodegenerative disorders, and AIDS (3). Apoptosis is caused by the activation within cells of a family of cysteine proteases, which specifically cleave their substrates at aspartic acid residues. These proteases are known as “caspases.” The Bcl-2 family proteins appear to control the “decision” step of apoptosis, determining whether certain caspases will or will not become activated (4–6). Antiapoptotic members of the Bcl-2 family such as Bcl-2 and Bcl-xL tend to prevent activation of these terminal effector proteases, whereas proapoptotic members Bax and BAK facilitate caspase activation.

Bax is the first proapoptotic homologue of the Bcl-2 family, which was identified by immunoprecipitation with the Bcl-2 protein (7). Overexpression of Bax accelerates cell death induced by a wide range of cytotoxic insults, whereas loss of Bax expression has been observed in a wide variety of human cancers and also contributes to poor response to chemotherapeutic drugs (8). Activation of this proapoptotic protein appears to involve intracellular translocation and homodimerization (9). Apoptotic stimuli induce a conformational change of the Bax protein, resulting in exposure of its N and C termini that appears to be required for the cytosolic Bax protein to move to the membranes of mitochondria where it inserts as a homodimer (10–12).

Evidence has accumulated that mitochondria play an important role in the control of apoptosis (13). Cytochrome c resides in the intermembrane space of mitochondria of healthy cells. Once released from mitochondria, cytochrome c binds to and activates Apaf-1, a human homologue of the Caenorhabditis elegans cell death protein CED-4 (14). Activated Apaf-1 then forms complexes with pro-caspase 9, resulting in caspase activation and apoptosis induction. Overexpression of proapoptotic Bcl-2 family proteins Bax, BAK, and BID induces cytochrome c release through a Bcl-2 suppressible mechanism (4). Thus, one possibility is that Bax may form selective channels for cytochrome c release from the inter-membrane space of mitochondria into the cytosol, although exactly how mitochondrial apoptogenic molecules escape during apoptosis remains controversial.

To gain further insights into Bax action, we performed a yeast two-hybrid screening to identify proteins that can bind to Bax. Here we describe the molecular cloning and functional characterization of a novel protein, termed Bif-1 for Bax-interacting factor-1, which interacts physically with the Bax protein and influences cell life and death.

EXPERIMENTAL PROCEDURES

Plasmids—The human Bif-1 cDNA in pG4–5 was obtained from a yeast two-hybrid screening by using LexA-Bax (JTMT) as bait. After digestion with EcoRI and XhoI, the Bif-1 cDNA was subcloned into the yeast two-hybrid vector pEG202 (15) or the mammalian expression vector PK-SFFV that was generated by replacing the CMV promoter of pCMV3.1 (Invitrogen, Carlsbad, CA) with the SFFV-LTR promoter from the SFFV-neo plasmid (16). The Bif-1 open reading frame was cloned in-frame into the EcoRI/XhoI-digested vector pEGFP-C2 (CLONTECH Lab, Inc., Palo Alto, CA), pGEX-1 (Amersham Pharmacia Biotech, Inc., Piscataway, NJ), or the EcoRI/SalI-digested pFLAG-CMV2 vector (17). All other plasmids have been described (18).

Yeast Two-hybrid Assays—Two-hybrid screens were performed essentially as described (15) in Saccharomyces cerevisiae EGY48 cells.
with plasmid pEG202 encoding LexA-Bax (ΔTM), fusion protein (19), and human fetal brain cDNA library cloned into pJG4–5 (CLON-TECH). Candidate clones were isolated from yeast colonies formed on leucine-deficient agar plates with detectable β-galactosidase activity and retested by cotransformation with the bait expressing plasmids.

**Northern Blot Analysis—** A human 12-lane multiple tissue Northern blot (CLONTECH) was hybridized at 68 °C overnight in Church buffer (0.5 M NaPO₄, pH 7.1, 2 mM EDTA, 0.1% sodium pyrophosphate, 7% SDS) containing 100 µg of single-stranded DNA and a [32P]dCTP-labeled probe (1.8 × 10⁹ cpm/ml) generated from an 0.8-kilobase N-terminal Bif-1 cDNA as template and random primers (Life Technologies, Inc., Baltimore, MD).

**Cell Transfections and Apoptosis Assays—** FL5.12 cells were maintained in interleukin-3 (IL-3)-containing medium and transfected with 25 µg of pK-SFFV-Bif-1 or parental vector DNA (Neo) by electroporation as described (20). 293 or 293T cells were transfected by a calcium phosphate method. Stable transfectants were selected by 1 mg/ml G418. The cell viability was determined by trypan blue dye exclusion, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Thiazolyl blue) assay, and flow cytometric analysis.

**Coimmunoprecipitation Assay—** Cells were lysed in Nonidet P-40 lysis buffer (20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 1.5 mM MgCl₂, 1 mM EDTA, 0.2% Nonidet P-40) or 3-[((3-cholamidopropyl)dimethylammonio)-1-propanesulfonic acid (Chaps) lysis buffer (150 mM NaCl, 10 mM Heps, pH 7.4, 1% Chaps) containing 1 mM phenylmethylsulfonyl fluoride, 5 µg/ml leupeptin, 1 µg/ml pepstatin A, and 5 µg/ml aprotinin. Immunoprecipitation and immunoblot assays were performed as described (18) with the indicated antibodies. The anti-Bif-1 polyclonal antiserum was generated in rabbits using glutathione S-transferase-Bif-1 fusion protein as immunogen. The glutathione S-transferase-Bif-1 protein was produced in *Escherichia coli* DH5α cells and purified by glutathione-Sepharose according to manufacturer’s recommendations (Amersham Pharmacia Biotech).

**Immunofluorescence Analysis—** Cells were cultured with 25 nM Mitotracker CM(II)ros (Molecular Probes, Eugene, OR) for 30 min prior to fixation in 4% paraformaldehyde and permeabilization in 0.5% Triton X-100. Immunofluorescent staining was performed as described (18) with anti-FLAG M2 monoclonal antibody (Sigma, St. Louis, MO), anti-Bax N20 rabbit antiserum (Santa Cruz, CA), or anti-Bif-1 monoclonal antibody (Imgenex Corp., San Diego, CA), which was detected by either fluorescein isothiocyanate (FITC)-conjugated or rhodamine-conjugated secondary antibodies (Chemicon International, Inc., Temecula, CA).

**RESULTS**

**Molecular Cloning of cDNAs for Bif-1—** To identify cDNAs encoding proteins that can bind to Bax, we performed a yeast two-hybrid screening using a LexA-Bax as the bait. A screen of 1.5 million independent transformants from a human brain Matchmaker cDNA library yielded 68 clones positive for both GAL1-LEU2 and GAL1-lacZ reporter gene expression. Of these, 3 clones (numbers 3, 8, and 21) encoded the same protein, designated Bif-1, for Bax-interacting factor-1. The rest of the clones were single clones, the sequences of which were not identifiable or encoded artificial proteins. Surprisingly, no Bcl-2 family members were found in this yeast two-hybrid screen, although they are no doubt expressed in the human brain cDNA library used and previously have been shown to interact with the Bax protein. All three Bif-1 cDNAs contained an open reading frame encoding a predicted protein of 365 amino acids (Fig. 1).

*Fig. 1.* Bif-1 is an evolutionarily conserved SH3 domain-containing protein. The predicted amino acid sequence of human Bif-1 protein and the homologous mouse, *C. elegans*, and *D. melanogaster* proteins are aligned, with identical residues in boxes. The predicted SH3 domain is indicated in bracket.
in cell-cell communication (21, 22). Bif-1, however, does not contain any of the conserved Bcl-2 homology (BH) domains of the Bcl-2 family proteins. We also performed BLAST searches to identify additional previously undescribed homologues of Bif-1 in mouse and possibly in the nematode C. elegans and the fruit fly Drosophila melanogaster. As shown in Fig. 1, the mouse Bif-1 protein contains 365 amino acids and shares 96% overall amino acid identity with human Bif-1, as deduced from expressed sequence tag clones AA615579, AA517877, AA592742, and AI159401. The C. elegans Bif-1 (AAB52640) is 366 amino acids in length and shares 42% identity and 59% similarity with the human Bif-1 protein. The D. melanogaster gene (AAF57578) encodes a protein of 426 amino acids that shares 39% identity and 57% similarity with human Bif-1.

**Tissue Distribution of Bif-1**—Northern blot analysis was used to assess the expression of Bif-1 mRNA in various human tissues. Hybridization with a Bif-1 probe revealed expression of Bif-1 in most tissues, with abundant expression in heart, skeletal muscle, kidney, and placenta (Fig. 2). Interestingly, three different size transcripts were detected for Bif-1, with major mRNAs of 1.5, 2, and 6 kilobases; it remains to be determined whether these different size transcripts arise from alternative splicing mechanisms and whether they encode different proteins.

**Bif-1 Associates with Bax in Yeast and Mammalian Cells**—Yeast two-hybrid analysis indicated that Bif-1, whether fused to a B42 transactivation domain (AD-Bif-1) or a LexA DNA-binding domain (LexA-Bif-1), strongly interacted with Bax, as determined by assays of β-galactosidase activity (Fig. 3A). The only region in the Bif-1 protein that shares significant amino acid homology to other known proteins is an SH3-like domain located between residues 308 and 364 of the human Bif-1 protein. To explore whether binding of Bif-1 to Bax requires this domain, a C-terminal deletion mutant of Bif-1 (residues 1–284) that lacks the SH3-like domain was expressed as a LexA-fusion protein and tested for its ability to interact with AD-Bax in budding yeast. As shown in Fig. 3A, LexA-Bif-1 (1–284) interacted with AD-Bax to a degree comparable with the interactions between full-length LexA-Bif-1 and AD-Bax. In contrast, AD-Bax failed to form two-hybrid interactions with LexA-Bif-1 (285–365), a deletion mutant of Bif-1 protein that essentially contained only the SH3-like region, indicating that the SH3-like domain of Bif-1 is not required for its interaction with Bax.

To confirm that association of Bif-1 and Bax can occur in mammalian cells, 293T cells were transiently transfected with expression plasmid encoding FLAG-tagged Bif-1 or the same parental vector lacking Bif-1 cDNA. Immunoprecipitates were prepared using anti-FLAG antibody and subjected to SDS-polyacrylamide gel electrophoresis (PAGE) immunoblot analysis using anti-Bax antibodies, revealing that endogenous Bax protein can coimmunoprecipitate with FLAG-Bif-1 (Fig. 3B). In addition, immunoblot analysis of the total lysates indicated that overexpression of FLAG-Bif-1 has no effect on Bax protein expression in 293T cells (Fig. 3B). More importantly, endogenous Bax could be coimmunoprecipitated with endogenous Bif-1 from the murine hematopoietic cell line FL5.12 (Fig. 3C), providing further evidence that the Bax-Bif-1 interaction occurs in the presence of physiological protein levels. Immunoprecipitations performed using preimmune serum (Fig. 3C) or empty vector (Fig. 3B) transfection confirmed the specificity of these results.

**Bif-1 Colocalizes with Bax in Cytosol**—To determine the in-
tracellular localization of Bif-1, we expressed Bif-1 as a green fluorescent protein (GFP) fusion protein in 293 epithelial cells. Fluorescence confocal microscopic analysis revealed an extranuclear distribution of GFP-Bif-1 (Fig. 4A). Two-color analysis using a mitochondrial-specific dye MitoTracker showed that a proportion of the GFP-Bif-1 protein molecules was associated with mitochondria (Fig. 4Ac). Similar results were obtained for endogenous Bif-1 protein in FL5.12 cells by immunofluorescence staining with anti-Bif-1-specific monoclonal antibody (Fig. 4, B and C). Upon induction of apoptosis by IL-3 withdrawal, Bif-1 was concentrated in punctate foci in the cytosol (Fig. 4, Bd and Cc), suggestive of association with mitochondria or other organelles. Double immunostaining with antibodies specific for Bax and Bif-1 indicated that Bif-1 was partially colocalized with Bax in the cytosol of FL5.12 cells (Fig. 4C).

Because certain apoptotic stimuli trigger Bax translocation from the cytosol to the membranes of mitochondria, it was important to determine whether Bif-1 affects the intracellular translocation of Bax in response to apoptotic signals. For this purpose, 293 cells were transiently cotransfected with GFP-Bax and FLAG-Bif-1 and treated without or with apoptosis-inducing agents, including staurosporine, vinblastine, and anti-Fas antibody. As shown in Fig. 5, GFP-Bax was located diffusely in untreated cells but became concentrated in the cytosol following apoptosis induction. This is consistent with previous studies (10, 23). Cells labeled with anti-FLAG monoclonal antibody revealed a mostly punctate distribution of FLAG-Bif-1 protein in the cytosol when coexpressed with the proapoptotic protein Bax. Consistent with the immunofluorescence data in FL5.12 cells, FLAG-Bif-1 was partially found in the same cellular compartments with GFP-Bax in 293 cells. Interestingly, the cytosolic redistribution of GFP-Bax in response to apoptotic stimuli was not observed in some cells that failed to receive the FLAG-Bif-1 plasmid DNA (Fig. 5, D and G), implying that Bif-1 may contribute to Bax translocation to mitochondria during apoptosis.

**IL-3 Deprivation Induces Bax Association with Bif-1**—To determine whether apoptotic stimuli-induced changes in Bif-1 colocalization with Bax correlate with alterations in Bax heterodimerization with Bif-1, we performed coimmunoprecipitation experiments. As shown in Fig. 6A, increased association of Bax with Bif-1 in FL5.12 cells was evident at 12 h after IL-3 deprivation and reached a maximum at 18 h. After 24 h of IL-3 withdrawal, the association between Bax and Bif-1 decreased (Fig. 6A), and more than half of the FL5.12 cells died (not shown). Immunoblot analysis using whole cell lysates showed that the protein levels of Bif-1 also slightly decreased following IL-3 withdrawal. Moreover, deprivation of IL-3 induced a conformational change in Bax as demonstrated by immunoprecipi-
tation with anti-Bax 6A7 monoclonal antibody that specifically recognizes the conformationally changed Bax protein (24, 25). This correlated closely with the protein complex formation between Bif-1 and Bax in response to growth factor withdrawal.

To determine whether Bax conformational change is required for its binding to Bif-1, we compared the ability of Bax to heterodimerize with Bif-1 under the presence of Nonidet P-40 versus Chaps. It has been reported that nonionic detergents such as Nonidet P-40 and Triton X-100 can cause a conformational change in Bax, whereas the zwitterionic detergent Chaps keeps Bax in its native conformation (24). FL5.12 cells were cultured with or without IL-3 for 16 h and lysed in lysis buffer containing either 1% Chaps or 0.2% Nonidet P-40. Immunoprecipitation was performed with anti-Bax polyclonal antiserum or preimmune serum as control, followed by immunoblot analysis of the resulting immune complexes with antibodies specific for Bax or Bif-1. In addition to immune complexes, the lysates (30 μg of protein) were analyzed directly.

To study the significance of Bif-1 for regulation of apoptosis, we stably transfected FL5.12 cells with expression plasmid encoding human Bif-1 or the same parental vector lacking Bif-1 as a control (Neo). FL5.12 cells are murine lymphoid progenitor cells that die via apoptosis in the absence of IL-3, thus providing a model commonly used to investigate the mechanisms of programmed cell death. Immunoblot analysis of lysates prepared from the resulting polyclonal bulk-transfected cell lines showed that levels of Bif-1 protein were markedly elevated in Bif-1-transfected FL5.12 cells, compared with FL5.12 cells that received the Neo control plasmid (Fig. 7B). These FL5.12 cells were then cultured for various times in medium without IL-3, and cell viability was assessed based on the ability to exclude trypan blue dye. The kinetics of cell death was markedly accelerated in cultures of IL-3-deprived Bif-1-expressing FL5.12 cells compared with FL5.12-Neo control cells. As shown in Fig. 7A, for example, only 40% of FL5.12 cells expressing Bif-1 remained viable at 12 h after growth factor withdrawal, compared with nearly 75% of FL5.12-Neo cells. These results were confirmed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide Thiazolyl blue assay (Fig. 7C). Consistent with previous studies (20), overexpression of Bcl-2 protected FL5.12 cells from apoptosis induced by IL-3 withdrawal.

To further characterize the feature of cells undergoing apoptosis, we performed flow cytometric analysis of the dead cells after staining with annexin V, a Ca\(^{2+}\)-dependent phospholipid-binding protein that binds to apoptotic cells with exposed plasma membrane phospholipid phosphatidylserine. As shown
buffer containing protease inhibitors. Cell lysates were normalized for presence or absence of IL-3 for 10 h and lysed in Nonidet P-40 lysis A applied directly to SDS-PAGE/immunoblot analysis.

The conformationally changed Bax protein was immunoprecipitated with anti-Bax rabbit antiserum. Also, the total lysates were prepared with Chaps lysis buffer containing protease inhibitors. The conformationally changed Bax protein was immunoprecipitated with anti-Bax 6A7 antibody and detected by immunoblotting with anti-Bax rabbit antiserum. Also, the total lysates were applied directly to SDS-PAGE/immunoblot analysis.

in Fig. 7D, 79% of Bif-1-transfected FL5.12 cells were annexin V-positive, compared with 66% of FL5.12-Neo cells when cultured for 12 h in the absence of IL-3. Moreover, caspase activation was examined by immunoblot analysis with antibodies specific for caspase-3 (Imgenex), cleaved D4-GDI (Imgenex), or tubulin (Sigma) control protein. B, FL5.12 transfectants were cultured with or without IL-3 for 8 h, and cell lysates were prepared with Chaps lysis buffer containing protease inhibitors. The conformationally changed Bax protein was immunoprecipitated by anti-Bax 6A7 antibody and detected by immunoblotting with anti-Bax rabbit antiserum. Also, the total lysates were applied directly to SDS-PAGE/immunoblot analysis.

in Fig. 7D, 79% of Bif-1-transfected FL5.12 cells were annexin V-positive, compared with 66% of FL5.12-Neo cells when cultured for 12 h in the absence of IL-3. Moreover, caspase activation was examined by immunoblot analysis with antibodies specific for caspase-3 or caspase-cleaved D4-GDI fragment. When deprived of IL-3, the protein levels of pro-caspase-3 dramatically decreased in FL5.12 cells overexpressing Bif-1 compared with FL5.12-Neo control cells (Fig. 8A). Cleavage of D4-GDI demonstrated further caspase activation in FL5.12-Bif-1 transfectants following IL-3 withdrawal (Fig. 8A).

In addition, we also investigated Bax conformational change in FL5.12 cells expressing Bif-1 after IL-3 withdrawal by immunoprecipitation with 6A7 Bax monoclonal antibody. When cultured in the presence of IL-3, no or very little conformationally changed Bax was detected in the 6A7 immunoprecipitates from both FL5.12-Bif-1 and FL5.12-Neo transfectants (Fig. 8B, lanes 1 and 3). In contrast, 8 h after deprivation of IL-3, a large proportion of Bax was immunoprecipitated with 6A7 antibody in FL5.12-Bif-1 cells (Fig. 8B, lane 4) compared with FL5.12-Neo control cells (Fig. 8B, lane 2).

**DISCUSSION**

Using a yeast two-hybrid screening approach, we have identified cDNAs encoding a novel Bax-binding protein, Bif-1, which is highly conserved throughout evolution. All members of the Bcl-2 family of proteins contain at least one of four evolutionarily conserved domains: BH1, BH2, BH3, and BH4, which can be important for their function and protein-protein interactions (26). Bif-1 interacts physically with Bax but lacks identifiable similarity to all four of the conserved BH domains, indicating that it is not a member of the Bcl-2 protein family.

The predicted amino acid sequence of Bif-1 contains an SH3-like domain at residues 308–364 and shares significant similarity to several SH3-containing proteins, including endophilin and GRB2-like proteins. The SH3 domain, which contains ~60 amino acids, binds to proline-rich sequences in many intracellular proteins. These protein-protein interactions regulate the cellular localization of protein-tyrosine kinases and their substrates such as those involved in signaling at the cell surface or regulating the cytoskeleton (22). Recently, these interactions also are implicated in the regulation of apoptosis (27, 28). The SH3 domain-containing protein SETA binds to AIP1/Alix, apoptosis-linked gene 2 (ALG-2)-interacting protein 1 or ALG-2-interacting protein X, and sensitizes astrocytes to UV-induced apoptosis (27). Similarly, overexpression of dynamin-2, an SH3-interacting GTPase, triggers apoptosis in a p53-dependent manner (28). Moreover, the deletion mutant of dynamin-2 lacking the proline/arginine-rich domain triggers apoptosis more potently than the wild-type, suggesting that the SH3-binding domain mediates negative regulation of an apoptotic activity in the dynamin-2 protein (28).

We have identified a novel SH3 domain-containing protein, Bif-1, which also participates in regulating apoptosis possibly through activating the Bax-mediated cell death pathway. Bax resides largely in the cytosol of healthy cells, despite the presence of a typical TM domain near its C terminus (10, 29). Apoptotic stimuli cause a conformational change in Bax, inducing its translocation and integration into the membranes of mitochondria and promoting apoptosis (10, 11, 30). Our studies indicate that Bif-1 binding to Bax may contribute to induction of Bax conformational change in response to apoptotic signals. In murine hematopoietic cells, IL-3 withdrawal induced Bif-1 association with mitochondria and colocalization with Bax (Fig. 4). In addition, overexpression of Bif-1 in 293 cells seems to promote the translocation of Bax to mitochondria following apoptosis induction (Fig. 5). Moreover, deprivation of IL-3 induced increased association of Bax with Bif-1 in FL5.12 cells, which was accompanied by induction of Bax conformational change (Fig. 6). Interestingly, the nonionic detergent Nonidet P-40, which can induce a conformational change in Bax, dramatically reduced Bax heterodimerization with Bif-1 (Fig. 6B).

Based on these results, we propose a “hit-and-run” model for Bax-Bif-1 interaction that Bif-1 binds to the “inactive” form of Bax in the cytosol and induces a conformational change in this protein. Once conformationally changed or integrated into intracellular membranes, Bax no longer requires interaction with Bif-1. Indeed, overexpression of Bif-1 in FL5.12 cells promoted Bax conformational change, caspase activation, and apoptotic cell death following growth factor withdrawal. However, how apoptotic stimuli trigger the interaction of Bif-1 with Bax is unclear. One possibility is that the ability of Bif-1 to induce Bax conformational change is controlled by mechanisms of post-translational modifications. For example, the ability of Bad, a proapoptotic member of the Bcl-2 family, to heterodimerize with Bcl-xL to induce apoptosis is mediated by mechanisms controlling the state of phosphorylation of Bad (31, 32). In addition, cleavage of Bid, another proapoptotic member of the Bcl-2 family, by caspase-8 or granzyme B generates a truncated Bid fragment that binds to and induces Bax conformational change during apoptosis (33–36). Although additional work is clearly required to further study the molecular mechanism of Bax-Bif-1 interaction, the data shown here argue that Bif-1 may promote apoptosis by inducing a conformational change of Bax leading to its mitochondrial targeting.

**Acknowledgments**—We thank the Molecular Biology, Flow Cytometry, and Molecular Imaging core facilities at the H. Lee Moffitt Cancer Center and Research Institute for support.
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