Caspase cleavage of Bim<sub>EL</sub> triggers a positive feedback amplification of apoptotic signaling

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Members of the Bcl-2 protein family that share only the Bcl-2 homology 3 (BH3) domain are known mostly as sentinels for apoptotic stimuli and initiators of apoptosis. One BH3-only protein, Bim, is the major physiological antagonist of the prosurvival proteins in B and T lymphocytes. It is required for hematopoietic homeostasis and to preclude autoimmunity. Here, we show that the Bim<sub>EL</sub> isoform, which was predominant in T cells, existed in both phosphorylated and unphosphorylated forms. Whereas the unphosphorylated Bim<sub>EL</sub> was sequestered to microtubules by means of a direct interaction with tubulin, the phosphorylated protein was released from microtubules. The freed Bim<sub>EL</sub> was subjected to caspase cleavage at an early stage of apoptosis induced by stimuli that activate either the mitochondria- or death receptor-dependent apoptosis pathway. The N-terminally cleaved Bim<sub>EL</sub> became hyperactive in inducing apoptosis because of its more efficient targeting of Bcl-2. Thus, unlike many other BH3-only proteins, Bim<sub>EL</sub> can be activated downstream of the caspase cascade, leading to a positive feedback amplification of apoptotic signals.

A key step in the mitochondria-dependent apoptotic pathway is the disruption of the mitochondrial membrane. The integrity of the membrane is controlled primarily by a balance between the antagonistic actions of the proapoptotic and antiapoptotic members of the Bcl-2 family. The Bcl-2 homology 3 (BH3)-only family members constitute a key group of proapoptotic proteins that resemble Bcl-2 only in the BH3 domain. This domain is required for the interaction of these proteins with other Bcl-2 family members (1, 2). BH3-only proteins normally reside in other subcellular compartments or structures and translocate to the mitochondria in response to apoptotic stimuli. When at the mitochondria, they induce the conformational change and oligomerization of Bax and Bak, two Bcl-2 family members with BH1, BH2, and BH3 domains. The pore-forming capability of the oligomerized Bax and Bak results in the destabilization of the mitochondrial outer membrane and the subsequent release of the death molecules from the confines of the mitochondria (2). Antiapoptotic Bcl-2 family members, such as Bcl-2 and Bcl-xL, bind directly to the proapoptotic members, neutralize their activities, and abolish the apoptotic signaling. Given the pivotal role of the BH3-only proteins in sensing apoptotic stimuli and initiating apoptosis, their activities have to be kept in check to prevent inappropriate cell death (1).

Investigation into the diverse functions and regulations of the ∼10 known mammalian BH3-only proteins suggests that different proteins may initiate apoptosis in different cell types and/or transduce distinctive apoptotic stimuli in a given cell type (1, 3). Gene knock-out studies have revealed an essential role of one such protein, Bim, in shaping the development of the immune system and transducing the apoptotic signals caused by cytokine deprivation, calcium ion flux, and microtubule perturbation, but not other insults, in lymphocytes (4–7). Bim also facilitates HIV-1 Tat-induced apoptosis in T cells (8), which contributes in part to the progressive T cell depletion associated with AIDS. Bim is the major physiological antagonist of the prosurvival proteins, at least in B and T lymphocytes (5). It is essential for the development of T cells (5) and apoptosis of activated T cells (7). Here, we report a previously undescribed mechanism by which Bim controls the apoptotic signaling in T cells. Three main isoforms of Bim (Bim<sub>EL</sub>, Bim<sub>L</sub>, and Bim<sub>S</sub>) exist because of alternative splicing (9). The expressions of these isoforms vary in different cell types and tissues (10), with Bim<sub>EL</sub> being the predominant one in T cells. Our data indicate that Bim<sub>EL</sub> was sequestered to microtubules by means of a direct interaction with tubulin. Phosphorylated Bim<sub>EL</sub> (pBim<sub>EL</sub>) was released from microtubules and cleaved by caspases at an early stage of apoptosis, induced by stimuli that activate either the mitochondria- or the death receptor-dependent apoptotic pathway. The N-terminally cleaved Bim<sub>EL</sub> demonstrated a higher affinity for Bcl-2 and a markedly enhanced apoptotic activity. The activation of Bim<sub>EL</sub> downstream of the caspase cascade may provide a positive feedback amplification of apoptotic signals.

Methods

Cell Death Analyses. Apoptosis was induced in Jurkat T cells by 50 ng/ml tumor necrosis factor α (TNF-α)/0.5 µM staurosporine/0.05 µM taxol or by UV irradiation at a dose of 120 J/m². The broad-spectrum caspase inhibitor N-benzylxoycarbonyl-Val-Ala-Asp-fluoromethylketone was used at 20 µM. Cells were treated with 0.5 µM of the phosphatase inhibitor okadaic acid for 2 h. Cell death was measured by flow-cytometric analysis of hypodiploid nuclei stained with 20 µg/ml propidium iodide or annexin V-propidium iodide double stain, according to the manufacturer’s instructions (Roche Applied Science).

Phosphatase Treatment of Bim<sub>EL</sub>. Jurkat T cells (1 × 10⁶) were lysed in lysis buffer L containing 50 mM HEPES-KOH (pH 7.8), 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40, 2 mM DTT, and 0.5 mM PMSF. The lysate was dialyzed against 90 mM sodium citrate buffer (pH 4.8) containing 1 mM DTT, 1 mM PMSF, and the human protease inhibitor mixture (Sigma). After the addition of 1 unit of potato acid phosphatase (PAP; Sigma), the lysate was incubated at 30°C for 15 min and then analyzed.

In Vivo and in Vitro Binding Assays. For the in vivo Bcl-2-binding assay, constructs expressing the Flag-tagged wild-type or Bim<sub>EL</sub> were transfected into Jurkat T cells by electroporation. Cells

Abbreviations: BH, Bcl-2 homology; LC9, light chain 9; PAP, potato acid phosphatase; pBim<sub>EL</sub>, phosphorylated Bim<sub>EL</sub>; tBim<sub>EL</sub>, truncated Bim<sub>EL</sub>; TNF-α, tumor necrosis factor α.

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were lysed in the modified lysis buffer L, containing 500 mM NaCl, 2 days after transfection. Precleared lysate was incubated with the anti-Flag agarose beads (Sigma) at 4 °C for 2 h. After extensive washes in the same buffer, Flag-tagged BimEL and its associated Bcl-2 were eluted with the Flag peptide. The samples were subjected to Western blotting with anti-Bim and anti-Bcl-2 antibodies.

The in vitro tubulin-binding assay (8) was carried out by incubating 1 μg of purified tubulin (Sigma) with GST-BimL or GST-BimEL fusion proteins (1 μg) immobilized on glutathione agarose beads for 20 min at room temperature in 50 μl of binding buffer B (20 mM Tris, pH 7.5/500 mM NaCl/10% glycerol/0.2 mM EDTA/1 mM DTT/1 mM PMSF). After extensive washes with the same buffer, tubulin associated with GST-BimL or GST-BimEL was eluted with the SDS/PAGE sample buffer and detected by anti-tubulin Western blotting.

The microtubule-binding assay was performed essentially as described with some modifications (13). Jurkat cells (1 × 10^6) were lysed in the modified lysis buffer L, containing 500 mM NaCl, 2 days after transfection. Precleared lysate was incubated with the anti-Flag agarose beads (Sigma) at 4 °C for 2 h. After extensive washes in the same buffer, Flag-tagged BimEL and its associated Bcl-2 were eluted with the Flag peptide. The samples were subjected to Western blotting with anti-Bim and anti-Bcl-2 antibodies.

**Results**

Both Phosphorylated and Unphosphorylated BimEL Exist in Jurkat T Cells. To elucidate the mechanism of Bim-controlled apoptosis in the immune system, the regulation of Bim activity during apoptosis in T cells was investigated. In Jurkat T cells, Western blotting with the rabbit polyclonal antibodies directed against the full-length BimEL (a generous gift from X. Luo and X. Wang, Howard Hughes Medical Institute and University of Texas Southwestern Medical Center, Dallas) revealed a doublet with mobility similar to that of the Flag-tagged recombinant BimEL (rBimEL) in an SDS gel (Fig. 1A, lanes 1 and 3). The antibodies did not detect the other two Bim isoforms (BimL or BimS) in the lysate, although they are reactive with the rBimL and rBimS proteins (lane 2 and data not shown). Because phosphorylation often controls the activity of the Bcl-2 family members (14), we suspected that the doublet detected by immunoblotting represented the phosphorylated and unphosphorylated forms of BimEL. In support of this idea, incubation of Jurkat cell lysate with PAP converted the upper band of the doublet into the lower one (Fig. 1A, lanes 4 and 5). In contrast, treatment of Jurkat cells with the phosphatase inhibitor okadaic acid produced the opposite effect of turning the lower band into the upper one. This was confirmed by immunoblotting with the rabbit polyclonal antibodies directed against the full-length BimEL (10). These results indicate that phosphorylation of BimEL is involved in regulating its activity in Jurkat cells.

**Activated Mouse Primary T Cells.** T cell suspensions from female C57BL/6 mice lymph nodes were cultured in RPMI medium supplemented with 10% FBS in plates coated with 5 μg/ml anti-CD3 and 1 μg/ml anti-CD28 for 2 h.

**Discussion**

The regulation of Bim activity during apoptosis in T cells was investigated. In Jurkat T cells, Western blotting with the rabbit polyclonal antibodies directed against the full-length BimEL (a generous gift from X. Luo and X. Wang, Howard Hughes Medical Institute and University of Texas Southwestern Medical Center, Dallas) revealed a doublet with mobility similar to that of the Flag-tagged recombinant BimEL (rBimEL) in an SDS gel (Fig. 1A, lanes 1 and 3). The antibodies did not detect the other two Bim isoforms (BimL or BimS) in the lysate, although they are reactive with the rBimL and rBimS proteins (lane 2 and data not shown). Because phosphorylation often controls the activity of the Bcl-2 family members (14), we suspected that the doublet detected by immunoblotting represented the phosphorylated and unphosphorylated forms of BimEL. In support of this idea, incubation of Jurkat cell lysate with PAP converted the upper band of the doublet into the lower one (Fig. 1A, lanes 4 and 5). In contrast, treatment of Jurkat cells with the phosphatase inhibitor okadaic acid produced the opposite effect of turning the lower band into the upper one. This was confirmed by immunoblotting with the rabbit polyclonal antibodies directed against the full-length BimEL (10). These results indicate that phosphorylation of BimEL is involved in regulating its activity in Jurkat cells.

**Conclusion**

The results presented in this study suggest that phosphorylation of BimEL is involved in regulating its activity in Jurkat cells. Further studies are needed to determine the exact mechanism of Bim-controlled apoptosis in the immune system.
upper one (lanes 6 and 7). Thus, the upper band most likely contained the pBimEL.

Caspase Cleavage of pBimEL at an Early Stage of Apoptosis. Interestingly, treatment of Jurkat cells with several apoptosis stimuli, such as the cytotoxic drug staurosporine, UV irradiation, and the death-inducing cytokine TNF-α, resulted in the disappearance of the upper band and the appearance of a new faster-migrating band of ~22 kDa (Fig. 1A, lanes 11, and 13). This effect was completely blocked by N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (lanes 10, 12, and 14), a broad-spectrum caspase inhibitor, suggesting that the ~22-kDa fragment was produced by a caspase-cleaved product of pBimEL (tBimEL). To confirm the specificity of the polyclonal antibodies used in immunoblotting, the antibody solution was preincubated with the purified pBimEL before its incubation with the membrane. This process completely abrogated the detection of both the doublet and the ~22-kDa fragment (Fig. 1A, lanes 19 and 20), indicating that these proteins indeed originated from BimEL. It is worth noting that the anti-Bim antibodies purchased from StressGen Biotechnologies and ProMab Biotechnologies detected neither the pBimEL nor the cleaved tBimEL (Fig. 1A, lanes 21–24).

A time-course analysis revealed that tBimEL began to appear in Jurkat T cells 1 h after the treatment with staurosporine, at which time only ~6% cells were undergoing apoptosis (Fig. 1B). The caspase cleavage of BimEL, at an early stage of apoptosis, suggested that tBimEL was not merely an end product generated in dead cells but, rather, may contribute to the execution and progression of apoptosis from the beginning.

Different from staurosporine, UV, and TNF-α, both the microtubule-perturbation drug taxol and the HIV-1 Tat protein, which was shown recently to induce apoptosis by disrupting microtubule dynamics (8), caused not only the caspase-dependent cleavage but also a caspase-independent modification of BimEL. This finding was illustrated by the appearance of a slow-moving BimEL species in the gel (mBimEL; Fig. 1A, lanes 15, 16, and 18). The nature and significance of this modification remain to be investigated. We suspect that it may be responsible for the observed strong dependence on Bim for both taxol- and Tat-induced apoptosis in T cells (5, 8).

Finally, it is important to point out that the caspase cleavage of the pBimEL was not restricted to Jurkat T cell line. Induction of apoptosis in activated mouse primary T cells by staurosporine differed dramatically from that of Jurkat cells (Fig. 1C).

In Vitro Cleavage of pBimEL by Recombinant Caspase-3. Because the apoptotic stimuli activating either the mitochondria pathway (staurosporine, UV, taxol, and Tat) or the death receptor pathway (TNF-α) were all capable of inducing the cleavage of pBimEL, the downstream effector caspses that are common to both pathways were probably responsible for this effect. Consistent with this idea, incubation of recombinant caspases-3 (Calbiochem) with the lysate of healthy Jurkat cells yielded a cleaved pBimEL fragment that comigrated with the tBimEL generated in vivo from apoptotic Jurkat cells (Fig. 1D). This finding further confirmed the 22-kDa fragment as a caspase-cleaved product of pBimEL.

Caspase Cleavage of BimEL after Asp-13. To determine the cleavage site in BimEL, a series of N- and C-terminal deletion mutants of BimEL, with truncations after several candidate Asp residues were produced and their mobility in an SDS gel was compared with that of the proteolytic fragment of pBimEL from apoptotic cells (Fig. 2A). Deletion of the first 7 or the last 21 amino acids from BimEL produced protein fragments that migrated slower than the caspase-cleaved 22-kDa tBimEL. tBimEL, however, comigrated with the N-terminal deletion mutant BimELΔN13 lacking amino acids 2–13 (Fig. 2A, lanes 2 and 3). Further deletion to the next Asp at position 53 or 157 produced much smaller BimEL fragments (data not shown). Further support for the existence in BimEL of a caspase-cleavage site after Asp-13 came from the observation that mutation of Asp-13 to Asn (D13N) in vitro had no effect (Fig. 2B).

Cleaved BimEL Has Greater Apoptotic Activity. BimEL has been identified as an apoptosis inducer. However, its potency is

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Cleaved BimEL Binds More Efficiently to Bcl-2. Bim has been shown to induce apoptosis by interacting with the prosurvival Bcl-2 family members through its BH3 region (9). Because ΔN13 demonstrated higher apoptotic activity than the full-length BimEL, we examined whether this was because of its increased binding to Bcl-2. Constructs expressing the Flag-tagged full-length BimEL and ΔN13 were electroporated into Jurkat T cells, and Bcl-2 associated with the immunoprecipitated BimEL proteins was analyzed by Western blotting. When the expressions of BimEL and ΔN13 were normalized to a similar level, the amount of Bcl-2-associated ΔN13 was significantly higher than that with the full-length BimEL (Fig. 2E). Thus, the N terminus of BimEL appeared to have an inhibitory effect on the BimEL–Bcl-2 interaction. The removal of the N-terminal 13 residues may expose the BH3 region for a more efficient binding to Bcl-2.

Direct Interactions of Tubulin and Microtubules with BimEL but Not BimL. We asked how, if only the pBimEL could undergo caspase cleavage during apoptosis, the phosphorylation of BimEL might control this process. The observation that the bacteria-produced pBimEL, which presumably was unphosphorylated, could be cleaved by caspase-3 in vitro (Fig. 2B) suggests that the BimEL phosphorylation was not essential for the cleavage process per se. This observation raises a possibility that the phosphorylation may release BimEL from a certain kind of sequestration, making BimEL accessible to caspase cleavage.

What sequesters the unphosphorylated BimEL and protects it from caspase cleavage? BimEL and BimL have been shown to interact with the dynein light chain 8 (LC8), which is part of the microtubular dynein motor complex (13). Apoptotic stimuli provoked the release of BimL and LC8, allowing BimL to associate with Bcl-2-like proteins (13). Although BimEL and BimL interact with LC8 (13) and Bcl-2 (9) with similar efficiency, BimL is much more apoptotic than BimEL (9). It is possible that BimEL is sequestered to microtubules more tightly than BimL by means of additional interactions, which may prevent its migration to the mitochondria.

In support of this hypothesis, purified tubulin (Sigma) was found to interact directly with immobilized GST-BimEL but not GST-BimL (Fig. 3A). An in vivo interaction of tubulin with Flag-tagged BimEL, but not Flag-tagged BimL, was also detected in anti-Flag immunoprecipitates derived from transfected 293T cells (Fig. 3B). These data indicate that BimEL, bound directly to tubulin, through a region (amino acids 42–97) that is missing from BimL.

To determine whether BimEL could also bind to polymerized microtubules, we performed a microtubule assembly assay in 293T cell lysate containing the transfected Flag-tagged BimEL or Flag-tagged BimL. The modified assembly conditions (see Methods) reduced the binding of motor proteins to microtubules and, as a result, the indirect docking of Bim onto microtubules through the motors. On ultracentrifugation to separate the polymerized microtubules and their associated proteins from the rest of the cell lysate, BimEL, but not BimL, was found to associate with microtubules (Fig. 3C).

pBimEL Escapes Sequestration by Microtubules. Microtubule-associated proteins (MAPs) interact with the negatively charged tubulin tails through their positively charged tubulin-binding regions. Phosphorylation of MAPs disrupts these interactions (15). We next asked whether, like other MAPs, pBimEL could dissociate from microtubules, leading to its cleavage by caspasess. First, microtubule-stabilizing drug taxol was added to cleared Jurkat cell lysate pretreated with or without PAP, or to cell lysate prepared from Jurkat cells treated with or without the phosphatase inhibitor okadaic acid. Ultracentrifugation was then performed to separate polymerized microtubules and their associated BimEL in the pellet from free tubulin and other soluble proteins in the supernatant. Both fractions, as well as the sample, were analyzed by Western blotting before ultracentrifugation (Fig. 4A, T). Without the PAP treatment, the majority of the unphosphorylated BimEL remained bound to microtubules in the pellet, whereas all of the pBimEL resided in the supernatant. After the PAP treatment, unphosphorylated BimEL migrated from the supernatant to the pellet to associate with the phosphorylated microtubules. In contrast to PAP, okadaic acid converted the unphosphorylated BimEL to the phosphorylated form, which dissociated from microtubules and stayed in the supernatant. Together, these two complementary approaches...
ultracentrifugation, microtubules. Recombinant caspase-3 was then added to the reaction. After a positive feedback apoptotic pathway involving the release of pBimEL from in vitro by caspase-3 blotting. (Fig. 4B) The association with microtubules prevented BimEL from cleavage. (Fig. 4D). The reaction was then subjected to ultracentrifugation to separate microtubules from free tubulin. Compared with only a weak cleavage of the microtubule-bound, unphosphorylated BimEL, a much more efficient cleavage of the free pBimEL was observed (Fig. 4B). This conclusion was supported further by the result obtained in staurosporine-treated Jurkat cells, in which only the pBimEL released from microtubules was cleaved (Fig. 4C).

Discussion

Bim is the major physiological antagonist of the prosurvival proteins in B and T lymphocytes (5). It functions by maintaining hematopoietic homeostasis and precluding autoimmunity (5). By performing experiments in Jurkat and activated mouse primary T cells, in which expression of the BimEL isoform is predominant, we have discovered a mechanism by which BimEL participates in the control of apoptosis (Fig. 4D). BimEL was found to exist in both the phosphorylated and unphosphorylated forms in healthy cells, and the unphosphorylated form was sequestered to microtubules by means of a direct interaction with tubulin. This interaction depended on a region in BimEL that is missing in BimL, allowing BimEL to be more tightly sequestered to microtubules and, consequently, less available for Bcl-2 binding than BimL. This finding may explain why BimEL is generally less apoptotic than BimL, despite the fact that both can bind to the dynein LCS (13) and Bcl-2 (9) with similar efficiency.

Phosphorylation of BimEL caused it to dissociate from microtubules. The freed BimEL, however, was mostly inactive because it displayed a low affinity for Bcl-2 (Fig. 4D). On the induction of apoptosis, the pBimEL released from microtubules was processed by a caspase(s) to generate an N-terminally truncated fragment (AN13) with an increased affinity for Bcl-2 and a significantly enhanced apoptotic activity. Because apoptotic stimuli activating either the death receptor pathway (TNF-α) or the mitochondria pathway (UV, staurosporine, Taxol, and HIV-1 Tat) are all capable of inducing the cleavage of BimEL, it is likely that a caspase(s) that is common to both pathways is responsible for this event. Although caspase-3 has been shown to cleave BimEL in vitro, it is important to point out that the caspase(s) that is in charge in vivo remains to be elucidated.

What could the caspase cleavage of BimEL contribute to the overall control of apoptosis in T cells? Several pieces of data suggest that the cleavage is not required for the initiation of apoptosis. First of all, the induction of apoptosis did not cause any enhancement in BimEL phosphorylation (as reflected by similar levels of unphosphorylated BimEL in healthy and apoptotic cells; Fig. 4C) or release of BimEL from microtubules (Fig. 4C, compare lanes 1 and 3). More importantly, the cleavage of BimEL depended on the activation of the downstream caspases. Although the cleavage of BimEL does not trigger the onset of apoptosis, it may in fact contribute to a positive feedback amplification of apoptotic signals (Fig. 4D). This idea is supported by the demonstration that the cleaved tBimEL occurred very early during the course of apoptosis and was unlikely to be a simple end product generated in dead cells (Fig. 1B). Furthermore, tBimEL was more active than the full-length protein in targeting Bcl-2 and inducing apoptosis (Fig. 2). It is worth noting that this signal-amplification mechanism initiated by the cleav-

Fig. 4. pBimEL escapes sequestration by microtubules and becomes accessible to caspase cleavage. (A) pBimEL is released from microtubules. Jurkat cell lysate preincubated with or without PAP (left) or lysate from Jurkat cells treated with or without okadaic acid (right) was incubated with taxol and then subjected to ultracentrifugation. α-Tubulin and Bim in the pellet (P), supernatant (S), or lysate before centrifugation (T) were detected by Western blotting. (B) The association with microtubules prevents BimEL from cleavage by caspase-3 in vitro. Jurkat cell lysate was incubated with taxol to assemble microtubules. Recombinant caspase-3 was then added to the reaction. After ultracentrifugation, α-tubulin and Bim were analyzed as in A. (C) pBimEL is released from microtubules and cleaved by caspases in apoptotic Jurkat cells. Lysate from Jurkat cells treated with or without staurosporine (Stau) was subjected to the same analysis as in A. (D) Diagram depicting the activation of a positive feedback apoptotic pathway involving the release of pBimEL from microtubules and caspase cleavage of pBimEL. The cleaved tBimEL(AN13) binds more Bcl-2 and has greater apoptotic activity. tBimEL may or may not contain the phosphorylation sites, which have not been mapped in the full-length BimEL. See the text for details.

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age of BimEL seems to apply only to mature T cells, whereas in immature T cells undergoing apoptosis, an increased expression of BimEL and BimL has been observed (6). It is unclear what causes such a change in BimEL regulation and whether this change is biologically significant for T cell development.

It is interesting to note that although the signal-amplification effect caused by caspase cleavage of the pBimEL is different from the reported role of the BimL isoform in sensing and initiating the apoptosis pathway (13), similarities can be detected in the control of the activities of the two isoforms. In human breast carcinoma cell line MCF-7 and mouse IL-3-dependent promyelocytic cell line FDC-P1, certain apoptotic stimuli can lead to the phosphorylation of BimL by the Jun N-terminal kinase (16) and the subsequent release of BimL from the sequestration by the microtubular dynein motor complex. This process enables BimL to translocate to the mitochondria and target Bcl-2 or its homologues to initiate apoptosis (13).

The signal-amplification function of the caspase-cleaved BimEL is similar to a previously described amplification activity displayed by another BH3-only protein Bid. Induction of death receptor-initiated apoptosis triggers the activation of caspase-8, which cleaves the inactive cytosolic form of Bid into a truncated Bid (tBid). tBid then translocates to mitochondria and displays higher affinity for antiapoptotic Bcl-2 (17, 18). Whereas the Bid-mediated amplification may be more restricted to the death receptor pathway, BimEL appears to function in a more general manner to amplify death signals initiated from both the mitochondria- and the death receptor-pathways. Future experiments are needed to shed more light on the posttranslational regulation of BimEL and the biological consequences of the BimEL-mediated signal-amplification process.

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