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Ubiquitin-mediated Degradation of the Proapoptotic Active Form of Bid

A FUNCTIONAL CONSEQUENCE ON APOPTOSIS INDUCTION*

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Kristin Breitschopf, Andreas M. Zeiher, and Stefanie Dimmeler‡

From the Division of Molecular Cardiology, Department of Internal Medicine IV, University of Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany

Under basal conditions, the proapoptotic protein Bid is a long-lived protein. Pro-apoptotic stimuli such as tumor necrosis factor-α (TNFα) or Fas induce its caspase-8-mediated cleavage into two fragments. The COOH-terminal cleavage fragment of Bid (tBid) becomes localized to mitochondrial membranes and triggers the release of cytochrome *c*. Here we show that tBid is ubiquitinated and subsequently degraded by the 26 S proteasome. Degradation of tBid is significantly inhibited by the proteasome inhibitors MG-132 and lactacystin. In contrast, caspase-specific or lysosomal inhibitors do not affect tBid stability. Furthermore, mutation of the putative ubiquitin acceptor sites within tBid results in a stabilized protein as assessed by pulse-chase analysis. To address whether tBid degradation might be regulated by interaction with other Bcl-2-like proteins, cotransfection studies were performed. However, neither the presence of proapoptotic Bax nor antiapoptotic Bcl-2 or Bcl-XL affected tBid degradation. Finally, we determined the functional role of tBid degradation. Overexpression of stabilized tBid proteins significantly enhanced cytochrome *c* release and subsequent apoptosis induction approximately 2-fold compared with wild type tBid. Similarly, tBid-induced apoptosis was considerably amplified by inhibition of tBid degradation using the proteasome-specific inhibitor MG-132. Thus, proteasomal degradation of tBid limits the extent of apoptosis in living cells.

Programmed cell death is critical for the successful development of multiple tissues and the maintenance of normal tissue homeostasis. The signaling pathways involved in apoptosis have been extensively studied (1–6). Key regulatory proteins in apoptotic events are the Bcl-2 family of proteins, which can either improve cell survival (Bcl-2, Bcl-XL, A1, Mcl-1, Bcl-W) or promote cell death (Bax, Bak, Bcl-XS, and Bok) (2). These molecules are characterized by the presence of several conserved motifs, described as the Bcl-2 homology domains (BH domains) [1] (7). The domains are required for the intermolecular association of Bcl-2 protein family members. A new set of proapoptotic Bcl-2 protein family members that possess only one conserved Bcl-2 homology domain, the death-promoting BH3 domain, has been recently identified. These proteins are called the "BH3 domain-only" molecules, which include Bid, Bik, Bad, Hrk, Bim, Blk, and EGL-1 of *Caenorhabditis elegans* (8–14).

The BH3 domain-only protein Bid is localized in the cytosolic fraction of cells as an inactive precursor (15, 16). Its active form is generated upon proteolytic cleavage by caspase-8 in response to treatment with TNFα or anti-Fas. Human Bid is cleaved at aspartic acid 60 after the LQTD site, and thereby, a truncated COOH-terminal cleavage fragment is produced, referred as tBid (Fig. 1). tBid translocates to mitochondria and induces cytochrome *c* release. Thereby, Bid relays an apoptotic signal from the cell surface to mitochondria (15–17). However, the precise molecular mechanism responsible for the translocation of tBid onto mitochondrial membranes and for the subsequent release of cytochrome *c* from mitochondria to cytosol during apoptosis is still unknown.

In addition to transcriptional regulation, protein stability of Bcl-2 members also plays an important regulatory role for cell survival. For instance, interleukin-3 withdrawal- or Fas-induced caspase-mediated cleavage of Bcl-2 considerably affects susceptibility to apoptosis in Jurkat or Ba/F3 cells (18). Furthermore, Bcl-2 degradation is a key regulatory event in the execution of TNFα-mediated apoptosis in endothelial cells (19). In these cells, TNFα induces the dephosphorylation and subsequent ubiquitin-dependent degradation of Bcl-2 (20). On the other hand, less is known about the role of stability of proapoptotic Bcl-2 family members in the regulation of apoptosis. It has been reported that proapoptotic Bax is cleaved by calpain in HL60 cells induced to undergo apoptosis (21). However, blockage of Bax cleavage did not affect the execution of apoptosis. Alternatively, the Bax protein has been shown to accumulate in the presence of the proteasomal inhibitor MG-132, implicating the ubiquitin proteolytic system in controlling Bax stability (22).

The ubiquitin-proteolytic pathway is a major system for selective protein degradation in eukaryotic cells. One of the first steps in this process includes selective modification of ϵ-NH_2 groups of lysine residues in the corresponding protein by ubiquitination, which targets the protein for ubiquitin-dependent degradation by the proteasome complex. Although the mechanisms that underlie this multicatalytic process are very well characterized (23–26), the signals that target proteins for ubiquitination and, therefore, determine their stability are often unclear. In some cases, different patterns of phosphorylation or

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‡ To whom correspondence should be addressed: Div. of Molecular Cardiology, Dept. of Internal Medicine IV, University of Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany. Tel.: 49-69-6301-7440; Fax: 49-69-6301-7113; E-mail: Dimmeler@em.uni-frankfurt.de.

[1] The abbreviations used are: BH domain, Bcl-2 homology domain; TNFα, tumor necrosis factor-α; tBid, cleaved COOH-terminal fragment of Bid; wt, wild type; HA, hemagglutinin.

a partially conserved sequence motif, as shown for mitotic cyclins, other cell cycle regulators, or transcription factors, are required (26).

The objectives of this study were to identify whether induction of apoptosis is affected by alterations in Bid/tBid stability and to get further insights into the biological function and mechanism of tBid-triggered apoptosis. We demonstrate here that tBid is degraded rapidly by the ubiquitin proteolytic system. Stabilization of tBid either by proteasomal inhibitors or by inactivation of putative ubiquitin acceptor amino acid residues enhances cytochrome *c* release and subsequent apoptosis in HeLa cells. Therefore, the life span of tBid appears to act as an important regulatory element in Bid-dependent apoptotic pathways.

EXPERIMENTAL PROCEDURES

Cell Culture—COS-7 and HeLa cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum and 2 mM L-glutamine.

Plasmid Constructs—Human wild type (wt) Bid and the COOH-terminal fragment tBid were amplified by polymerase chain reaction with oligonucleotides containing *Eco*RI and *Bam*HI restriction sites and cloned into the respective sites of pcDNA3.1(−)MycHis under the transcriptional control of the cytomegalovirus promoter (Invitrogen, Groningen, The Netherlands). Desired mutant tBid constructs were obtained by site-directed mutagenesis (Stratagene, Heidelberg, Germany). Human Bax or Bcl-XL were cloned by polymerase chain reaction with oligonucleotides containing *Kpn*I and *Hin*dIII or *Apa*I and *Sac*I restriction sites, respectively, and subcloned into the respective sites of pcDNA3.1(−) (Invitrogen, Groningen, Netherlands). Human Bcl-2 was amplified and subcloned into pcDNA3.1(-)MycHis (Invitrogen, Groningen, Netherlands) as described recently (19). Plasmid encoding HA-tagged human ubiquitin was cloned into pcDNA3.1(−). Sequences were determined using an ABI automated sequencer.

Transient Transfection System—Transient transfections of COS-7 cells were performed as described previously (19). HeLa cells were transfected with plasmids using the calcium phosphate method (27) or the FuGENE 6 transfection reagent (Roche Molecular Biochemicals). Transfection with FuGENE 6 was carried out according to the instructions of the manufacturer (3.5×10^5 cells/6-cm well; 3 μg of plasmid DNA, 6 μl of FuGENE 6 transfection reagent).

Western Blot Analysis and Immunoprecipitation—Cells were lysed as described (19). Western blots were either performed with anti-Bid antibody (Santa Cruz Biotechnology, Santa Cruz, CA), anti-myc-antibody (Santa Cruz Biotechnology), or anti-cytochrome *c* (Pharmingen, San Diego, CA).

To identify ubiquitinated forms of tBid, HeLa, or COS-7 cells, transiently cotransfected with various myc-tagged tBid constructs and HA-tagged ubiquitin in pcDNA3.1(−), were incubated with the proteasome inhibitor lactacystin (10 μM) for 2 h (28). Cells were lysed, and protein concentrations were determined by the Bradford method (29). Equal amounts of protein were subjected to immunoprecipitation with anti-myc antibody. The immunoprecipitates were resolved via 12.5% SDS-polyacrylamide gels and transferred onto polyvinylidene difluoride membrane, and the conjugates were detected using anti-HA antibody (Santa Cruz Biotechnology) and the enhanced chemiluminescence (ECL) method (Amersham Pharmacia Biotech). As a control for expression of myc-tagged Bcl-2 protein, Western blot analysis was performed with an anti-myc antibody (Santa Cruz Biotechnology).

Preparation of Mitochondria—42 h after transfection, HeLa cells were scraped off the plates and pelleted by centrifugation at $800 \times g$ for 10 min. Then cells were resuspended in hypotonic lysis buffer (20 mM HEPES, pH 7.5, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride) and incubated for 3 min on ice. Cells were homogenized with 25 strokes in a dounce homogenizer and centrifuged at $750 \times g$ for 15 min at 4 °C to remove nuclei and unbroken cells. The supernatant was recovered and centrifuged at $10{,}000 \times g$ for 15 min at 4 °C, and the mitochondrial pellet was resolved in 25 μl of lysis buffer.

Stability of Proteins in Vivo—HeLa or COS-7 cells were starved in

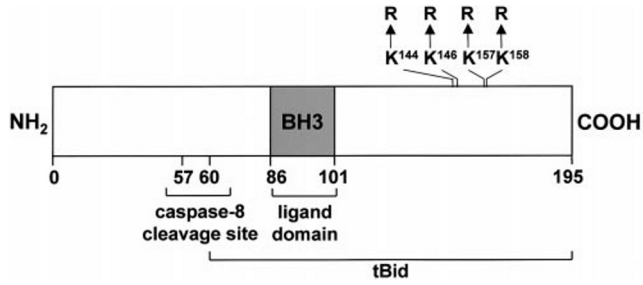

FIG. 1. **Schematic diagram of Bid.** Homologue domains (*BH*) are indicated by *hatched boxes*. The putative caspase-8 cleavage site within tBid is indicated. *Arrows* indicate amino acid substitutions made in this study. *K*, Lys/lysine; *R*, Arg/arginine. *tBid*, COOH-terminal fragment of Bid generated by cleavage with caspase-8.

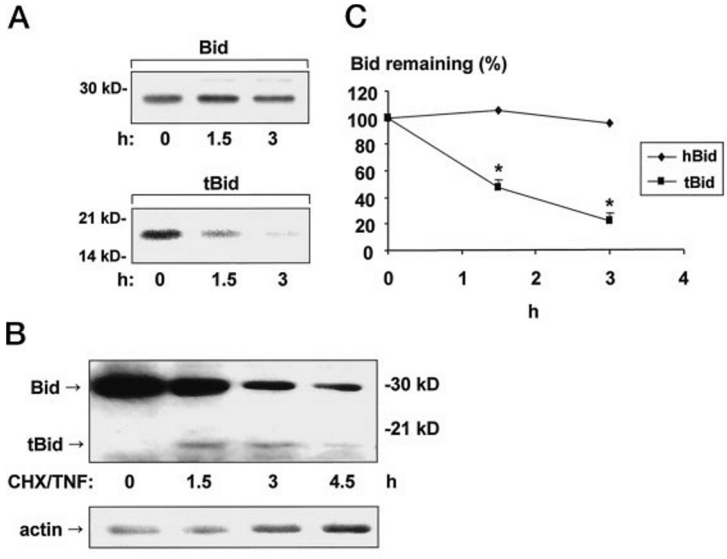

FIG. 2. **Stability of Bid and tBid *in vivo*.** *A*, ^{35}S-metabolically labeled Bid and tBid proteins were chased as described under "Experimental Procedures" and immunoprecipitated from aliquots containing equal amounts of proteins. A representative autoradiogram of three independent experiments is shown. *B*, quantitative (phosphorimaging) analysis of the data depicted in *A* are shown. Quantities are relative to the amount of protein at time 0. Data are the mean ± S.E. *, $p < 0.01$ *versus* amount of wt tBid protein at 0 h. *C*, representative time course of the degradation of tBid generated *in vivo*. Cells were incubated with TNFα (100 ng/ml) and cycloheximide (*CHX*; 10 μg/ml) for the time periods indicated. Formation of tBid protein was detected by Western blot analysis with anti-myc antibody. Similar results were obtained using an anti-Bid antibody. After stripping of the polyvinylidene difluoride membrane, equal loading of the samples was demonstrated by Western blot analysis with anti-actin antibody.

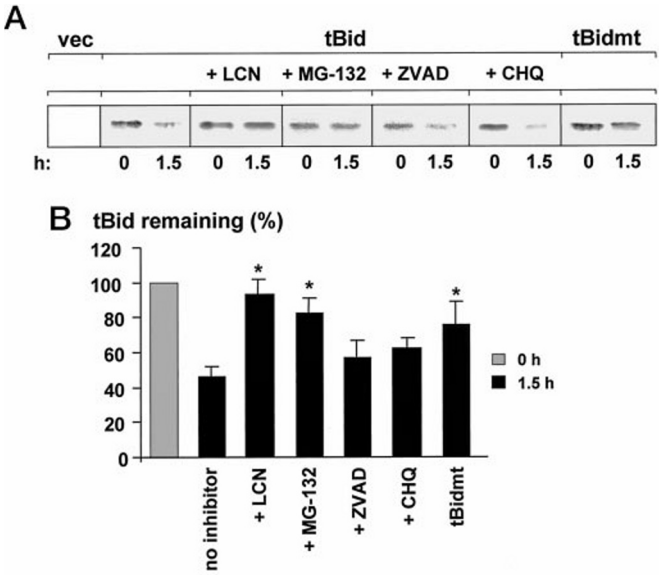

FIG. 3. **Sensitivity of tBid proteins to various protease inhibitors.** *A*, COS-7 cells transiently transfected with tBid pcDNA3.1 were pulse-labeled (2 h) with [^{35}S]methionine and [^{35}S]cysteine and chased (1.5 h) in the absence or presence of various protease inhibitors (lactacystin (*LCN*), 10 μM; MG-132, 100 μM; benzyloxycarbonyl-Val-Ala-DL-Asp-fluoromethylketone (*ZVAD*) 100 μM; chloroquine (*CHQ*), 100 μM) as described under "Experimental Procedures." *tBidmt*, lysine-free tBid construct in which Lys-144, -146, -157, and -158 were mutated to Arg; *vec*, empty vector. A representative autoradiogram is shown ($n = 3$). *B*, quantitative analysis of three independent experiments described in *A* after a chase period of 1.5 h. Quantities are relative to the amount of protein at time 0. Data are the mean ± S.E. (*, $p < 0.05$ *versus* amount of wt tBid protein after 1.5 h chase; $n = 3$).

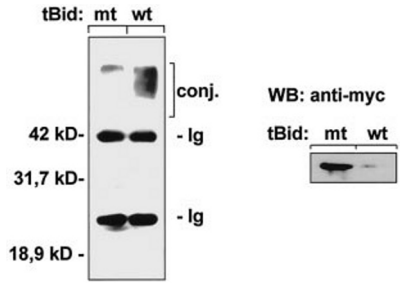

FIG. 4. **Detection of ubiquitin-tBid conjugates in COS-7 cells.** COS-7 cells were transiently cotransfected with expression vectors containing either a lysine-free myc-tagged tBid construct (*tBidmt*) or myc-tagged wt tBid and HA-tagged ubiquitin. 42 h after transfection, cells were incubated for additional 2 h with the proteasome inhibitor lactacystin. Equal amounts of protein, as determined by the Bradford method (29), were subjected to immunoprecipitation with anti-myc antibody and ubiquitin conjugates were identified using Western blot (*WB*) analysis and anti-HA antibody. Expression of tBid protein is detected by Western blot analysis with anti-myc antibody. *Conj.* denotes conjugates, and *Ig* indicates the heavy and light chain of the Ig molecule.

Dulbecco's modified Eagle's medium without methionine and cysteine for 1 h, then metabolically labeled with L-[^{35}S]methionine and L-[^{35}S]cysteine for 2 h. Cells were then chased in nonradioactive medium for the time periods indicated. Cells were lysed (10 mM Tris-HCl, pH 8, 1% Triton X-100, 0.32 M sucrose) at 4 °C for 20 min. Samples containing equal amounts of protein were immunoprecipitated with an anti-myc antibody. Immunocomplexes were collected using immobilized protein A/G-plus Sepharose (Amersham Pharmacia Biotech) and resolved on 15% SDS-polyacrylamide gel electrophoresis. The gel was dried, and proteins were visualized by a PhosphorImager (Molecular Dynamics).

For the assay of tBid generation *in vivo* and its subsequent degradation, cells were incubated for the time periods indicated with 100 ng/ml TNFα and 10 μg/ml cycloheximide. Cell extracts were obtained by lysis of cells in 10 mM Tris-HCl, pH 8, 1% Triton X-100, and 0.32 M sucrose on ice for 20 min. Then homogenates were centrifuged, and the resulting supernatant was used for Western blotting. Proteins (30 μg/lane) were resolved via 15% SDS-polyacrylamide gel electrophoresis and probed with anti-Bid or anti-myc antibody (Santa Cruz Biotechnology), and ECL was performed according to the instructions of the manufacturer (Amersham Pharmacia Biotech).

Cell Death Analysis—DNA fragmentation was demonstrated and quantified by morphological analysis of apoptotic nuclei after fluorescence staining with 4′,6-diamidinophenylidole as described previously (19). To determine the influence of the various tBid constructs on apoptosis, HeLa cells were transiently cotransfected with β-galactosidase reporter and test plasmids. Cells were fixed in 4% formaldehyde, and transfected cells were identified by β-galactosidase staining. The percentage of morphologically altered cells typical for apoptotic cell death was determined by phase contrast microscopy and quantified as the number of total blue cells under each condition. Dead *versus* viable cells were counted by two blinded independent investigators in a total number of 600 cells.

Statistics—Data are expressed as mean ± S.E. from at least 3 independent experiments. Statistical analysis was performed with analysis of variance followed by modified LSD test (SPSS-Software).

RESULTS AND DISCUSSION

We previously showed that incubation with TNFα stimulates ubiquitin-dependent breakdown of the antiapoptotic protein Bcl-2 (19, 20). To identify other apoptosis-related proteins that might influence induction of apoptosis by alterations of protein stability in intact cells, we performed a functional analysis of the life span of the proapoptotic Bcl-2 member Bid and its active COOH-terminal fragment tBid (Fig. 1). COS-7 or HeLa cells were transiently transfected with either myc-tagged Bid or tBid constructs, and pulse-chase analyses were carried out. No significant difference in full-length Bid protein levels could be observed after 1.5 and 3 h chase following metabolic labeling of COS-7 cells (Fig. 2, *A* and *B*). In contrast, the COOH-terminal fragment tBid was rapidly degraded, resulting in a half life of less than 1.5 h (Fig. 2, *A* and *B*).

Caspase-8-mediated cleavage of transiently transfected Bid was induced in intact cells by TNFα and cycloheximide and incubated for the time periods indicated. Whole cell extract containing all subcellular protein fractions was subjected to Western blot analysis. As can be seen in Fig. 2*C*, the tBid protein generated by caspase-8-mediated cleavage in intact cells does not accumulate. Rather, it appears to be rapidly degraded. Appropriate subcellular distribution of the transfected Bid and tBid constructs was confirmed by fluorescence microscopy of GFP fusion proteins in which Bid and tBid were tagged COOH-terminally to GFP and by subcellular fractionation (data not shown). Bid reveals a cytosolic localization, whereas tBid is exclusively localized to mitochondrial membranes.

tBid Is Rapidly Degraded by the Ubiquitin Proteolytic System—To characterize the system involved in tBid degradation, pulse-chase analyses were performed in the presence of various protease inhibitors. Neither the lysosomal inhibitor chloroquine nor the caspase-specific inhibitor benzyloxycarbonyl-Val-Ala-DL-Asp-fluoromethylketone affected tBid stability (Fig. 3). However, the proteasome inhibitors MG-132 and lactacystin significantly affected tBid stability (Fig. 3). Furthermore, a tBid construct in which all lysine residues that might act as potential ubiquitin acceptor amino acids were replaced with arginine (Fig. 1) exhibited a significantly extended life-span (Fig. 3).

To demonstrate that ubiquitin conjugates were intermediates in the degradation of tBid, COS-7 cells were transiently cotransfected with myc-tagged tBid and HA-tagged ubiquitin and then incubated with the proteasome inhibitor lactacystin. Fig. 4 illustrates the presence of highly ubiquitinated forms of tBid treated with lactacystin. Similar analysis with COS-7 cells transiently transfected with a tBid construct that lacks all

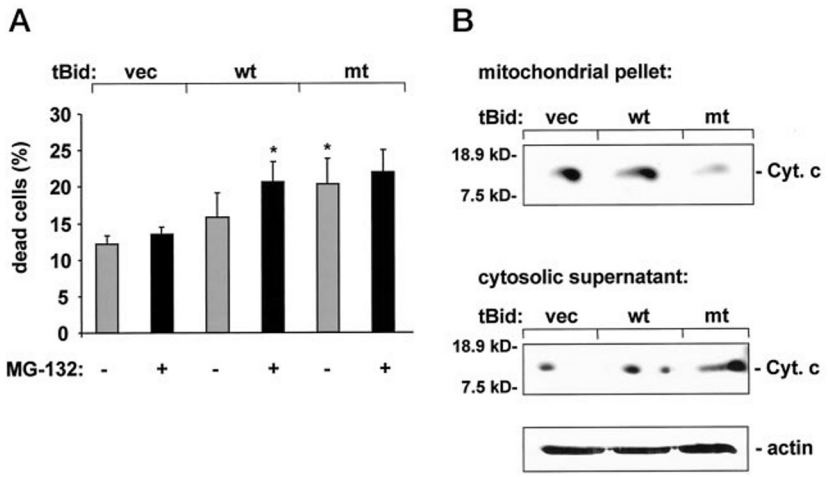

FIG. 5. **Effect of stabilized tBid proteins on apoptosis.** *A*, HeLa cells were transiently cotransfected with either empty vector, a vector carrying wt tBid, or a lysine-free tBid construct (*tBidmt*) and a lacZ reporter. 36 h following transfection, cells were incubated with or without the proteasome inhibitor MG-132 (100 μM) for the last 6 h of culture. Transfected cells were identified by β-galactosidase staining as described under "Experimental Procedures." Data are the mean ± S.E. (*, $p < 0.05$ *versus* wt tBid in the absence of MG-132; $n = 4$). *B*, release of cytochrome *c* from mitochondria. HeLa cells were transiently transfected with either empty vector, tBid, or tBidmt constructs. 42 h after transfection, cells were harvested, and mitochondria were isolated as described under "Experimental Procedures." Western blot analysis was carried out with anti-cytochrome *c* antibody. Equal loading of the samples was demonstrated by Western blot analysis with anti-actin antibody. *vec* denotes empty vector; *tBidwt*, wild type tBid; *Bidmt*, lysine-free tBid construct in which Lys-144, -146, -157, and -158 were mutated to Arg; *Cyt. c*, cytochrome *c*.

ubiquitin acceptor amino acids (lysine-free tBid or tBidmt) revealed less ubiquitin conjugates (Fig. 4). To demonstrate equal loading and expression of the various tBid constructs, Western blot analysis was carried out with an anti-myc antibody (Fig. 4). The lower intensity of the wt tBid band is due to its increased ubiquitinated forms. These data indicate that ubiquitin conjugates are formed during the degradation of the tBid protein.

A tBid Protein with an Extended Life Span Significantly Increases Apoptosis in HeLa Cells—To explore the functional role of tBid degradation, we examined the extent of apoptosis induction by wt tBid and degradation-resistant tBid constructs in intact cells. Stabilization of wt tBid by MG-132 resulted in a 2-fold increased rate of apoptosis of HeLa cells compared with HeLa cells in which tBid was not stabilized by MG-132 (Fig. 5*A*). In addition, expression of a degradation-resistant, lysine-free tBid construct resulted in a similar increase in apoptosis of HeLa cells (Fig. 5*A*). However, taking into account that the 26 S proteasome complex presents one of the major degradation systems within a cell, overall inhibition of this multi-protease complex could be rather unspecific, as stability of other proteins such as p53 or the inhibitor of NFκB (IκB) are also affected. Thus, it is well known that suppression of the proteasome function by proteasome-specific inhibitors promotes apoptosis (30, 31). Nevertheless, incubation of mock-transfected HeLa cells with MG-132 for 6 h did not significantly increase apoptosis in HeLa cells (Fig. 5*A*), suggesting that the increased apoptotic rate induced by MG-132 in wt tBid-transfected cells is due to the specific stabilization of wt tBid by MG-132. To verify that stability of tBid is indeed a regulatory event in apoptosis induction, the apoptotic rate of HeLa cells transiently transfected with a degradation-resistant, lysine-free tBid construct (tBidmt) was analyzed after treatment with MG-132 for 6 h. Only a slight difference of about 1.5% in the apoptotic rate of HeLa cells treated with MG-132 compared with HeLa cells that were not treated with MG-132 was observed (Fig. 5*A*). These data indicate a striking relevance of tBid stability in apoptosis induction.

Since tBid was shown to be the molecular adapter between the cytosolic, caspase-8-mediated apoptotic signaling pathway and the mitochondrial death machinery (15–17, 32), the direct influence of stabilized tBid protein on cytochrome *c* release was examined. Fig. 5*B* illustrates that stabilized tBid markedly increased cytochrome *c* translocation from mitochondria to the cytosol. These findings clearly indicate that inhibition of tBid degradation promotes apoptosis.

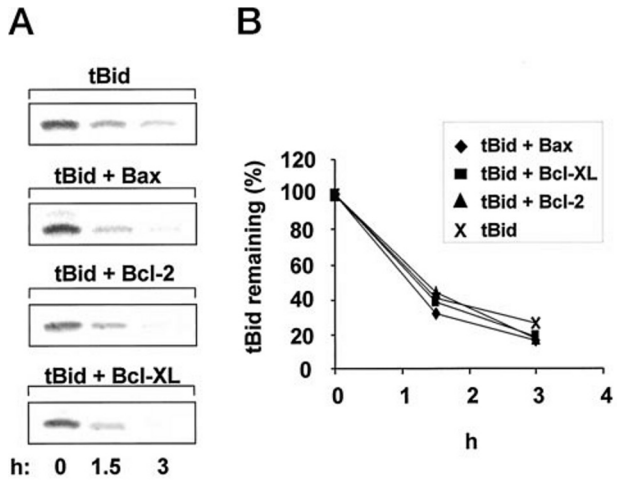

FIG. 6. **tBid is not affected by Bax, Bcl-XL, or Bcl-2.** *A*, COS-7 cells, transiently cotransfected with wt tBid and either Bax, Bcl-XL, or Bcl-2 constructs were pulse-labeled (2 h) with [^{35}S]methionine and [^{35}S]cysteine and chased for 0, 1.5, and 3 h as described under "Experimental Procedures." A representative autoradiogram is shown. *B*, Quantitative (phosphorimaging) analysis of the data depicted in panel *A* are shown. Quantities are relative to the amount of protein at time 0.

Taken together, the results of this study reveal the following novel findings: (i) Active proapoptotic tBid is an unstable protein that is ubiquitinated and subsequently degraded by the 26 S proteasome, whereas its inactive precursor Bid remains stable. Mutation of putative ubiquitin conjugation sites within tBid results in a stabilized protein, as assessed by pulse-chase analyses. Interestingly, we still observed a slight degradation of the lysine-free tBid construct. This might be due to the fact that a free and exposed NH_2 terminus can also act as a potential ubiquitin attachment site and, therefore, can stimulate ubiquitin-dependent degradation (28). tBid might be such a substrate for N-terminal ubiquitination. Structural analysis of the solution structure of Bid suggests a potential exposed BH3

domain after caspase-8-mediated Bid cleavage (33, 34). It is of note that this BH3 domain is located in the very N-terminal part of tBid underlining the existence of a free and exposed NH_2 terminus (Fig. 1). Nevertheless, this possibility has still to be tested. (ii) Overexpression of degradation-resistant tBid proteins significantly enhances tBid-induced cytochrome *c* release and subsequent apoptosis. Moreover, inhibition of tBid degradation in intact cells potentiates cell death (Fig. 5*A*). Therefore, one might speculate that the cell is capable of controlling the degradation of tBid, for instance by interaction with a target protein. There are several mitochondrial membrane-located Bcl-2 protein members such as Bcl-2, Bcl-XL, or Bax that are known to interact with tBid through its BH3 domain (10). Structural analysis of Bid suggests that caspase-8-mediated cleavage of Bid leads to an exposure of the BH3 death domain, thereby facilitating complex formation of tBid with other Bcl-2 members (33, 34). Therefore, we investigated whether these proteins might affect the half-life of tBid. COS-7 cells were transiently cotransfected with tBid and either Bax, Bcl-2, or Bcl-XL, and pulse-chase analyses were performed. However, these studies revealed that Bcl-2 family proteins such as Bcl-2, Bcl-XL, or Bax do not affect tBid degradation (Fig. 6). tBid might be regulated by cytosolic proteins such as 14-3-3, which was shown to regulate the activity of another BH3-containing protein, Bad (35). However, coimmunoprecipitation studies did not provide evidence for an interaction of tBid with 14-3-3 proteins (data not shown). It is of note that Bid does not contain the known sequence motif required for specific binding to 14-3-3 (36). Therefore, tBid may interact with an as yet unidentified target, and this protein-protein interaction may co-ordinate tBid degradation.

It is also possible that tBid degradation could be activated by classical posttranslational modifications such as phosphorylation or dephosphorylation as reported for Bcl-2 (19, 20). However, Bid does not contain any potential consensus sequences for kinases like the mitogen-activated protein (MAP) kinases ERK1/2 or Akt kinase, and so far, it is unknown whether Bid is phosphorylated at all.

In summary, our study suggests an important regulatory role of tBid stability for cell death. In response to death signals such as TNFα or anti-Fas, caspase-8-mediated cleavage of Bid results in activation of the proapoptotic function of Bid. The present data demonstrate that stability of the cleavage product tBid plays an important role for determining the extent of apoptosis in living cells via cytochrome *c* release. Degradation of tBid by the ubiquitin proteolytic system might therefore alter the extent of apoptosis within a cell and, moreover, may reduce apoptotic cell death. Although the mechanisms of regulating tBid degradation are still enigmatic, our data indicate an important protective role of the ubiquitin proteasome pathway in apoptosis induction by diminishing the level of the proapoptotic protein.

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