Critical and Functional Regulation of CHOP (C/EBP Homologous Protein) through the N-terminal Portion*

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C/EBP homologous protein (CHOP) is an endoplasmic reticulum stress-inducible protein that plays a critical role in the regulation of programmed cell death; however, the regulation of its function has not been well characterized. We have previously demonstrated that CHOP is regulated by the ubiquitin-proteasome system. In this study, during the process of clarifying the mechanism of the degradation of CHOP, we identified a novel regulation domain of CHOP in its N-terminal portion that is involved in various regulations and functions. The CHOP N-terminal domain is necessary not only for protein degradation but also for its transactivity and interaction with p300. In addition, trichostatin A, a histone deacetylase inhibitor, repressed the degradation of CHOP protein via the N-terminal domain. TRB3, a mammalian tribbles homolog that functions as a repressor of CHOP, also interacted with CHOP via the N-terminal portion and significantly blocked the association of p300 with CHOP. These results suggest that the N-terminal portion of CHOP plays a crucial role in its functional regulation and enable us to identify a novel function of TRB3 as an intracellular antagonist of the p300-binding domain of CHOP.

CHOP was originally isolated as a gene induced in response to DNA-damaging agents; subsequently it has been revealed that CHOP is induced by extracellular and endoplasmic reticulum (ER) stress (2, 3). ER stress responses are induced by cellular stresses, such as glucose starvation and hypoxia, that prevent protein folding and cause misfolded or malfolded proteins to accumulate in the ER (4, 5). Under such conditions, the homeostasis of protein folding in the ER is maintained by inter-organelle signaling from the ER to the nucleus, a process known as the unfolded protein response. Thus, from yeast to humans, the transcription of genes encoding molecular chaperones and folding enzymes in the ER is induced in the nucleus in response to unfolding in the ER, and excessive or long-term accumulations of unfolded proteins in the ER result in the apoptosis of cells. From experiments on the overexpression of its protein and knock-out mice, CHOP has been shown to act as an inducer of cell cycle arrest and apoptosis during ER stress. In addition, a recent study suggested that CHOP-mediated apoptosis during ER stress is implicated in diseases with ER stress-dependent cell death, such as neurodegenerative disease and/or type I diabetes (6).

During ER stress, CHOP is mainly induced via activation of the ER-localized kinase double-stranded RNA-activated protein kinase (PKR)-like ER kinase (PERK) through the downstream phosphorylation of a translation initiation factor, eukaryotic initiation factor 2α (eIF2α), and induction of a transcription factor, activation transcription factor 4 (ATF4) (7). Recently, it has been shown that four different kinases phosphorylate eIF2α and activate its downstream pathway, including induction of ATF4 and CHOP. These pathways are termed the integrated stress response, and each response is activated by a distinct upstream activation signal (7). Amino acid starvation activates the integrated stress response through general control non-derepressible-2 (GCN2) (8), while viral infection and iron deficiency activate the integrated stress response through the vertebrate-specific kinases double-stranded RNA-activated protein kinase (PKR) (9) and heme-regulated inhibitor kinase (HRI) (10), respectively, and stress from accumulation of unfolded or misfolded proteins in the ER activates the integrated stress response through PERK.

Because the basic region of CHOP is less conserved than that of other C/EBP family proteins, CHOP lacks DNA binding activity for the C/EBP-binding site. In addition, as CHOP cannot form homodimer, CHOP inhibits the ability of other C/EBP proteins such as NF-IL6 to bind DNA and exert transcriptional regulatory activity by forming heterodimers with their proteins (11). On the other hand, a CHOP-C/EBP heterodimer has been reported to bind to a unique DNA sequence, the CHOP-binding site, which is different from classical C/EBP-binding sites, and to act as a posi-

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2 The abbreviations used are: C/EBP, CCAAT/enhancer-binding protein; CHOP, C/EBP homologous protein; ATF4, activating transcription factor 4; ER, endoplasmic reticulum; HDAC, histone deacetylase; TSA, trichostatin A; aa, amino acid; GFP, green fluorescent protein; GST, glutathione S-transferase; E3, ubiquitin-protein isopeptide ligase.
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tive transactivator (12). In recent studies, several CHOP-inducible genes have been shown to be induced during ER stress via this CHOP binding sequence (13, 14). We very recently demonstrated that TRB3, a novel ER stress-inducible protein, is induced by CHOP with a novel dimerizing partner, ATF4, which is a transcription factor of ATF/CREB family member, via a novel CHOP binding sequence, CHOP-amino acid response element sites (15).

A large number of transcription factors undergo degradation via a ubiquitin-proteasome-dependent pathway (16, 17). A genetic study on Drosophila revealed that Sibo, a Drosophila homolog of C/EBP, is specifically degraded dependent on the expression of tribbles by the ubiquitin-proteasome (18). In humans, we have previously reported that C/EBP family transcription factors CHOP and Ig/EBP (C/EBPγ) are multiubiquitinated and subsequently degraded by the proteasome (19). TRB3, a human ortholog of tribbles, interacted with CHOP but did not promote degradation of CHOP protein. Therefore, the molecular mechanism involved in CHOP degradation is still unclear.

In this study, we identified the amino acid (aa) region required for degradation of the CHOP protein in its N-terminal portion. This region was also shown to be critical for CHOP transcriptional activity and interaction with p300; furthermore, TRB3 antagonized p300 binding to CHOP via this region. Degradation of CHOP protein via this region was suppressed by treatment with trichostatin A (TSA), and therefore this N-terminal domain of CHOP seemed to be crucial for various aspects of its functional regulation.

EXPERIMENTAL PROCEDURES

Reagents—RPMI 1640 medium, Dulbecco’s modified Eagle’s medium, anti-β-actin monoclonal antibody (AC-15), anti-FLAG monoclonal antibody (M2), trichostatin A, and tunicamycin were purchased from Sigma. Fetal bovine serum was from HyClone (Logan, UT). Anti-Myc monoclonal antibody (9E10) was from Roche Applied Science. A23187 was obtained from Calbiochem (La Jolla, CA). MG132 was obtained from Peptide Institute (Osaka, Japan). Cycloheximide was obtained from Nacalai Tesque (Kyoto, Japan). Anti-NF-IL6 polyclonal antibody (C-19) and anti-p300 polyclonal antibody (N-15) were from Novus Biologicals. Anti-p300 monoclonal antibody (N-15) was from Roche Applied Science. A23187 was obtained from Calbiochem (La Jolla, CA). MG132 was obtained from Peptide Institute (Osaka, Japan). Cycloheximide was obtained from Nacalai Tesque (Kyoto, Japan). Anti-NF-IL6 polyclonal antibody (C-19) and anti-p300 polyclonal antibody (N-15) were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-acetyl lysine polyclonal antibody (9441) was from Upstate Inc. (Lake Placid, NY). Anti-GFP monoclonal antibody (JL8) was from Clontech. The antisera against human CHOP was prepared by immunization of two female Japanese White rabbits (2–3 kg) with CHOP (1–169) protein fused with GST. The antisera against human TRB3 was prepared as described previously (15).

Cell Culture—Human melanoma cell line A375, human embryonic kidney cell line 293, and human hepatocellular carcinoma cell line HepG2 were cultured as described previously (19).

Construction of Expression Plasmids—The plasmids pcDNA3.1-Myc-CHOP, pcDNA3.1-Myc-CHOPSer79,82Ala, ΔBR, ΔLZ, ΔN9, ΔN18, Δ19–26, ΔN70, and pcCMV-FLAG-TRB3 were constructed as described previously (15, 19, 20). pcDNA3.1-Myc-CHOPA27–64, Δ37–64, S5/9A, T12A, S14/15A, Y22A, Y22F, and pcCMV-Gal4-CHOP, CHOPΔ19–26, pGEX6P-FLAG-CHOP, pGEX6P-TRB3 were generated by PCR. pcCMV-Gal4-CHOP WT, ΔN9, ΔN18, and ΔN70 were constructed as described previously (15). pMT-123 (HA-Ub) was kindly provided by Dr. D. Bohmann (European Molecular Biology Laboratory). pCMV-p300 was kindly provided by Dr. T. Okamoto (Nagoya City University, Nagoya, Japan). All constructs were verified by sequencing.

Reporter Gene Assays—Cells were transfected with luciferase reporter plasmids. After 48 h, lysates were prepared and luciferase assays were performed according to the manufacturer’s instructions (Promega). All experiments were performed a minimum of three times, and the values obtained were used to calculate means and standard deviations.

Recombinant Protein Expression—pGEX-6P-1 plasmids encoding the GST proteins alone or GST-TRB3 or GST-FLAG-CHOP fusion proteins were transformed into the BL21 strain of Escherichia coli (Novagen, Madison, WI). Protein expression and purification were performed according to the procedures outlined in the Bulk GST Purification Module (Amersham Biosciences). Recombinant FLAG-CHOP protein was prepared using PreScission Protease (Amersham Biosciences).

Immunoprecipitation and Western Blot Analysis—Cells were transiently transfected and treated as described in the figure legends. The cells were lysed in radioimmune precipitation buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% SDS, 0.5% deoxycholate, and 1% Triton X-100) supplemented with protease inhibitors. The lysates were subjected to immunoprecipitation as described in the figure legends. One to two percent of the lysates or co-immunoprecipitates were subjected to SDS-PAGE (12.5%), transferred onto polyvinylidene difluoride membranes, and probed with antibodies indicated in the figure legends. The immunoreactive proteins were visualized using ECL Western blotting detection reagents (Amersham Biosciences), and light emission was quantified with a LAS1000 lumino image analyzer (Fuji).

Transfection—A375 cells were transfected by a lipofection method using Effectene (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. 293 and HepG2 cells were transfected by the Chen-Okayama method (21).

RESULTS

Endogenous CHOP Protein Is Degraded by the Proteasome System—In normal conditions in multiple tissues and cells, the endogenous CHOP protein level is very low and its expression is induced at the transcriptional level during various extracellular and ER stresses. First, we investigated whether the level of endogenous CHOP protein is regulated through degradation by the proteasome during ER stress. We previously examined the effect of a proteasome inhibitor, MG132, on the endogenous CHOP protein level and showed that its expression was markedly augmented in the presence of MG132 (19). However, as the proteasome inhibitors induce ER stress by inhibiting degradation of unfolded proteins in the ER, there is a possibility that this augmentation was caused at the transcriptional level. Therefore, to directly assess whether CHOP protein is regulated by the proteasome system, we first examined the effect of MG132 on the rate of degradation of endogenous CHOP protein. The degradation rate was investigated by chasing with cycloheximide, a de novo protein synthesis inhibitor, in the presence of tunicamycin, a glycosylation inhibitor, for the indicated periods after pre-induction by treatment with tunicamycin.
cin for 6 h (Fig. 1, top). During ER stress induced by treatment with tunicamycin, endogenous CHOP protein level was effectively decreased by the degradation; however, NF-IL6 was not, as shown in a previous report (19). Similar results were obtained when CHOP was induced and chased by treatment with other ER stress inducers, A23187 and thapsigargin (data not shown). On the other hand, when chased by MG132, CHOP protein was significantly stabilized (Fig. 1, bottom). This repression of degradation was thought to be caused by the proteasome inhibitory action of MG132, thus confirming that endogenous CHOP induced during ER stress is degraded by the proteasome system.

The N-terminal Region of CHOP Is Critical for Degradation—During ubiquitination, ubiquitins are added to the lysine residues of target molecules through the association of specific ubiquitin ligases, E3. In addition, various modifications, such as phosphorylation, of substrates are necessary for the binding of E3 ligase(s) in some cases. Therefore, to investigate the mechanism of degradation of CHOP in detail, we constructed expression vectors for various mutants of CHOP and observed the expression level of these proteins. As shown in Fig. 2, CHOP deletion mutants truncated in the N-terminal region (CHOPΔN70 (aa 71–169) and CHOPΔN36 (aa 37–169)) were expressed at much higher levels compared with wild-type CHOP (Fig. 2A). On the other hand, other point and deletion mutants in the potential phosphorylation sites (Ser79, 82) and basic region, respectively, were expressed at levels similar to wild-type CHOP, and the deletion mutants of the leucine zipper domain were expressed at slightly lower levels (data not shown). To assess whether the augmentation of the expression of N-terminal-deleted CHOPs was caused by inhibition of degradation by the proteasome, we next examined the effect of MG132 treatment on the expression of these mutants. As shown in a previous report (19), the accumulation of wild-type CHOP protein was increased by treatment with MG132; however, CHOPΔN70 and CHOPΔN36 were not affected (Fig. 2B). These results suggest that the N-terminal region of CHOP is necessary for degradation by the proteasome.

We examined the subcellular localization of wild-type, ΔN70 (N-terminal deletion), or N70 (N-terminal alone) CHOP by using the GFP fusion system. GFP-CHOPΔN70 was primarily localized within the nucleus, as was wild-type CHOP; by contrast, GFP alone or GFP-CHOP N70 (aa 1–70) was detected in both the nucleus and cytoplasm (Fig. 2C). This result indicates that the nuclear localization signal of CHOP exists in the C-terminal region (aa 71–169) and the resistance to degradation of the N-terminal deletion mutants does not result from a difference of this subcellular localization compared with that of wild-type CHOP.

Identification of the Region Required for Degradation of CHOP—To further clarify the importance and function of the CHOP N-terminal region for degradation, we constructed additional N-terminal-truncated mutants (Fig. 3A). CHOPΔN18 (aa 19–169) and CHOPΔN19–26 (aa 1–18, 27–169) were highly expressed and were not affected by MG132 treatment, which was also the case for CHOPΔN70 and CHOPΔN36. On the other hand, CHOPΔ37–64 (aa 1–36, 65–169) showed almost the same basal expression level and enhancement of accumulation by MG132 as wild-type CHOP (Fig. 3B). CHOPΔN9 (aa 10–169) and CHOPΔ27–64 (aa 1–26, 65–169) were analyzed to visualize the subcellular localizations.

FIGURE 2. The N-terminal region of CHOP is necessary for degradation. A and B, A375 cells were transiently transfected with expression vector for wild-type Myc-CHOP or its mutants. After 24 h, the cells were treated with (B) or without (A) 2 μg/ml of tunicamycin/10 μM MG132 and 10 μg/ml of cycloheximide (CHX) for the indicated periods. The cell lysates were analyzed by immunoblotting using anti-CHOP, anti-NF-IL6, or anti-β-actin antibodies. C, A375 cells were transiently transfected with expression vector for GFP, wild-type GFP-CHOP, or its mutants. After 36 h, cells were analyzed to visualize the subcellular localizations.

FIGURE 1. Endogenous CHOP protein is degraded by proteasome activity. A375 cells were treated with 2 μg/ml of tunicamycin or 10 μM MG132 for 6 h and then chased with 2 μg/ml of tunicamycin/10 μM MG132 and 10 μg/ml of cycloheximide (CHX) for the indicated periods. The cell lysates were analyzed by immunoblotting using anti-CHOP, anti-NF-IL6, or anti-β-actin antibodies.
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In the ubiquitin-proteasome system, the level of degradation of many factors is controlled by the regulation of phosphorylation or dephosphorylation. These modifications act as switches for direct regulation, such as binding to E3 ligase(s), or indirect regulation, for example, by changing the subcellular localization. As the region between aa 10 and 26 of CHOP, which is required for the degradation, contains some potential sites for phosphorylation (Fig. 3C), we examined the effect of substitution of these amino acids with Ala on the sensitivity to the proteasome inhibitor. CHOP S5,9A, CHOP T12A and CHOP S14,15A protein levels were augmented by MG132 treatment (Fig. 3D). On the other hand, CHOP Y22A protein was accumulated under the normal conditions and was not affected by the proteasome inhibitor; however, the substitution of Tyr22 by Phe (CHOP Y22F) resulted in almost the same accumulation in response to MG132 treatment as wild-type CHOP (Fig. 3D). These results suggest that the phosphorylation or dephosphorylation of the CHOP N-terminal region is not significant for the constitutive degradation of CHOP but the Tyr22 amino acid residue plays an essential role in the degradation, probably due to its role in maintaining the N-terminal α-helix structure. In summary, we concluded that the N-terminal α-helix structure of CHOP is necessary for the degradation.

N-terminal-deleted CHOPs Are Not Polyubiquitinated—In the presence of MG132, CHOP protein was accumulated and multiubiquitinated, indicating that CHOP is constitutively ubiquitinated and degraded by the proteasome (19) (Fig. 2B). As the protein levels of N-terminal-deleted CHOPs were increased in the basal condition and were not affected by the treatment with proteasome inhibitor (Figs. 2 and 3), we examined whether these mutants are polyubiquitinated. Consistent with the protein stabilities, wild-type CHOP and CHOPΔN9 were highly and slightly, respectively, ubiquitinated upon co-expression with ubiquitin; however, CHOPΔN18 and CHOPΔ19–26 were not ubiquitinated under these conditions (Fig. 3E). CHOP Y22A was also

showed slightly lower expression levels as compared with the mutants insensitive to MG132, such as CHOPΔN18, and a slight increase of accumulation by MG132 treatment (Fig. 3B). These results indicate that the region between aa 10 and 26 in CHOP is required for degradation by the proteasome. As the main part of this region consists of an α-helix structure (Fig. 3A) (22), this motif may be significant for the binding to E3 ligase(s) or related molecule(s).

FIGURE 3. Identification of the region required of CHOP for degradation and ubiquitination. A and C, constructs of CHOP mutants and the accumulation of these mutants by MG132. B and D, A375 cells were transiently transfected with expression vector for wild-type Myc-CHOP or its mutants. After 24 h, the cells were treated with or without 2 μM MG132 for 12 h. The cell lysates were analyzed by immunoblotting using anti-Myc and anti-β-actin antibodies. The asterisk in the top panels indicates nonspecific bands. E, 293 cells were transiently transfected with the indicated constructs. After 24 h, the cells were treated with 10 μM MG132 for 12 h. The cell lysates were immunoprecipitated with anti-FLAG antibody, and multiubiquitinated CHOP was detected by immunoblotting with anti-HA antibody.

In the ubiquitin-proteasome system, the level of degradation of many factors is controlled by the regulation of phosphorylation or dephosphorylation. These modifications act as switches for direct regulation, such as binding to E3 ligase(s), or indirect regulation, for example, by changing the subcellular localization. As the region between aa 10 and 26 of CHOP, which is required for the degradation, contains some potential sites for phosphorylation (Fig. 3C), we examined the effect of substitution of these amino acids with Ala on the sensitivity to the proteasome inhibitor. CHOP S5,9A, CHOP T12A and CHOP S14,15A protein levels were augmented by MG132 treatment (Fig. 3D). On the other hand, CHOP Y22A protein was accumulated under the normal conditions and was not affected by the proteasome inhibitor; however, the substitution of Tyr22 by Phe (CHOP Y22F) resulted in almost the same accumulation in response to MG132 treatment as wild-type CHOP (Fig. 3D). These results suggest that the phosphorylation or dephosphorylation of the CHOP N-terminal region is not significant for the constitutive degradation of CHOP but the Tyr22 amino acid residue plays an essential role in the degradation, probably due to its role in maintaining the N-terminal α-helix structure. In summary, we concluded that the N-terminal α-helix structure of CHOP is necessary for the degradation.
TRB3 Inhibits the Association of CHOP with p300—Recently, TRB3, a human ortholog of tribbles, was identified as a novel Akt-binding and -regulating protein (23). We previously demonstrated that TRB3 is an ER stress-inducible protein and interacts with CHOP to inhibit its transcriptional activity (15). In addition, the region between aa 10 and 18 in CHOP is necessary for the interaction with TRB3 (15), indicating that this region is overlapping with the p300-binding site. Therefore, we examined the effect of TRB3 expression for the association of CHOP with p300. As shown in Fig. 5A, TRB3 expression dramatically inhibited the binding of CHOP with p300; on the other hand, p300 expression did not affect the binding of CHOP with TRB3. In our previous study, TRB3 also suppressed the transcriptional activity of ATF4, another ER stress-induced transcription factor (15). As shown in Fig. 5B, TRB3 inhibited the p300-ATF4 interaction as well. In an in vitro binding assay, recombinant GST-TRB3 dose-dependently interacted with recombinant FLAG-CHOP and inhibited the association of p300 with that (Fig. 5C). In addition, TRB3 expression suppressed coactivation of the transcriptional activity of wild-type CHOP by p300, whereas neither TRB3 nor p300 affected the transcriptional activity of CHOP ΔN18 (Fig. 5D). These results suggest that the affinity of the CHOP-TRB3 interaction is probably high as compared with that of the CHOP-p300 interaction and therefore TRB3 inhibits CHOP-dependent transcriptional activation by preventing CHOP-p300 association.

Trichostatin A Represses CHOP Protein Degradation—P300/CBP coactivators acetylate not only chromatin-conjugated histone but also various molecules such as transcription factor p53. The inhibition of cellular deacetylases leads to a longer half-life of endogenous p53; furthermore, p53 is ubiquitinated and acetylated on similar sites at the C terminus, suggesting that these modifications may compete for the same residues (24, 25). As CHOP bound strongly to p300, we next explored whether p300 acetylates CHOP and regulates its ubiquitination and degradation. First, we examined the effect of TSA, a histone deacetylase (HDAC) inhibitor, on the CHOP protein level and found that exogenous wild-type CHOP protein, but not ΔN18 or Δ19–26, accumulated in response to treatment with TSA as well as MG132 (Fig. 6A). The additive or synergistic effect of MG132 and TSA on the CHOP protein accumulation was not observed, suggesting that the effects of these treatments are caused by a similar mechanism. In addition, endogenous CHOP induced by tunicamycin was also accumulated as a result of TSA treatment in HepG2 cells (Fig. 6B) or A375 and 293 cells (data not shown). Furthermore, endogenous CHOP protein degradation was repressed in the presence of TSA (Fig. 6C). These results suggest that CHOP protein is stabilized by inhibition of HDAC activities. The stabilization of CHOP by TSA treatment was thought to be induced through the acetylation of CHOP protein. Therefore, we examined whether CHOP is acetylated by p300 in the presence of TSA or MG132. By Western blotting using an antibody specifically recognizing acetylated lysine residues, acetylated p53 was detected as a positive control; however, acetylated CHOP was not detected at all (Fig. 6D). This result suggests that the accumulation of CHOP protein induced by TSA is not caused through an antagonistic mechanism of acetylation for ubiquitination. On the other
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In this study, we have identified a novel functional domain of CHOP in the region between aa 10 and 26 that is necessary for not only the degradation of the protein but also its transcriptional activity and interaction with p300. Recently, there have been several reports showing that the acetylation of transcription factors can trigger critical regulation of activation or inactivation (26). CHOP associated with p300 but was not acetylated by it. Therefore, at least, p300 seems to not regulate the activity of CHOP via its acetylation. On the other hand, recently p300 was shown to be a crucial factor for degradation of p53 as E4 ligase (27). The N-terminal region of CHOP necessary for degradation was essential for interaction with p300; therefore, p300 may regulate the degradation of CHOP protein as well.

CHOP associated with MDM2, the E3 ligase for p53 (25), and Fbw1, the F-box protein for IkB (28) or β-catenin (29), in vivo (data not shown). The molecular mechanisms underlying whether these molecules function as the E3 ligase for CHOP are currently under investigation.

We showed here that CHOP degradation is repressed by the inhibition of deacetylation activity by TSA treatment via the region aa 10–26 as well. CHOP interacted with HDACs but was not acetylated upon the treatment with TSA, indicating that the degradation of CHOP is not regulated by its acetylation. Recently, it was revealed that the acetylation of MDM2 causes inactivation of its activity for degradation of p53 (30). Similarly, the E3 ligase for CHOP may be activated through deacetylation by HDAC(s) in untreated cells and may be inactivated through the inhibition of HDAC activity by TSA as well.

We previously showed that TRB3, an ER stress-induced kinase-like protein, associated with CHOP to suppress CHOP-dependent transcriptional activation through a proteolysis-independent pathway (15). TRB3 did not interfere with the dimerization of CHOP or with its DNA binding activity. In addition, as TRB3 repressed even the transcriptional activity of a GAL4 fusion protein of CHOP, it was suggested that TRB3 primarily inhibits CHOP transactivation, probably by inhibiting the modification of CHOP required for its transactivation or by interfering with the association of coactivator(s) or by recruiting corepressor(s) to DNA. Indeed, the TRB3 binding region of CHOP, aa 10–18, is a part of its p300 binding region, aa 10–26, and TRB3 expression significantly blocked the association of p300 with CHOP. TRB3 suppressed the transcriptional activity of ATF4, another ER stress-induced transcr-
tion factor. In the present study, TRB3 repressed the p300-ATF4 interaction as well. These results suggest a novel function of TRB3 as an intracellular antagonist of the p300-binding domains of both CHOP and ATF4. As TRB3 and other tribbles family members contain the classic substrate-binding domains of a protein kinase but not the ATP-binding and kinase-activa-
tion domains (31), TRB3 could act as a novel type of decoy kinase-like protein for Akt or other substrates. Similarly, TRB3 could act as a novel type of decoy deacetylase-like protein for p300 as well.

This study revealed that various molecules regulate the function of CHOP via effects on its degradation. CHOP is induced by various stress signals, such as ER stress, oxidative stress, hypoxia, and amino acid deprivation. Therefore, it is possible that CHOP signaling is strictly regulated by these stresses via its N-terminal portion. The analysis of these mechanisms may identify potential targets for the regulation of CHOP function.

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