Biochemical and Biophysical Research Communications **256,** 127–132 (1999)
Article ID bbrc.1999.0289, available online at http://www.idealibrary.com on IDEAL®

IκBα Ubiquitination Is Catalyzed by an SCF-like Complex Containing Skp1, Cullin-1, and Two F-Box/WD40-Repeat Proteins, βTrCP1 and βTrCP2

Hiroshi Suzuki,* Tomoki Chiba,† Masato Kobayashi,* Masahiro Takeuchi,* Toshiaki Suzuki,†,‡ Arata Ichiyama,‡ Tsuneo Ikenoue,† Masao Omata,§ Kiyoshi Furuichi,* and Keiji Tanaka†,[1]

**Molecular Medicine Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba-shi, Ibaraki, 305-8585, Japan; †Tokyo Metropolitan Institute of Medical Science, and CREST, Japan Science and Technology Corporation (JST), 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613, Japan; §Department of Gastroenterology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan; and ‡First Department of Biochemistry, Hamamatsu University School of Medicine, 3600 Handa-cho, Shizuoka, Hamamatsu 431-3192, Japan*

Received January 25, 1999

Destruction of the transcriptional inhibitor IκB by the ubiquitin (Ub) system is required for signal-dependent activation of the multifunctional transcriptional factor NF-κB, but details of this ubiquitination are largely unknown. We report here that the IκBα-ubiquitin ligase (IκBα-E3) is an SCF-like complex containing Skp1, cullin-1, and two homologous F-box/WD40-repeat proteins, βTrCP1 and βTrCP2. Intriguingly, all these components are cooperatively recruited to bind to a phosphorylated IκBα (pIκBα) produced by tumor necrosis factor-α (TNF-α) stimulation. IκBα-E3 bound to pIκBα catalyzed *in vitro* ubiquitination of pIκBα in the presence of ATP, Ub, and E1-activating and E2-conjugating enzymes. Forced expression of βTrCP1 and βTrCP2 resulted in dramatic augmentation of the *in vitro* polyubiquitination activity of IκBα-E3. These results indicate that the long-sought IκBα-E3 is an SCF-like complex consisting of multiple proteins which are coordinately assembled during phosphorylation of IκBα in response to external signals.

Ubiquitin (Ub) is a highly conserved 8.6-kDa polypeptide. The covalent modification of Ub has been implicated to play critical roles for various biologically important processes, such as cell-cycle regulation, signal transduction, immune and stress responses, and so on (1). The past decade's tremendous progress in understanding the ubiquitination system provides a new insight into the importance of proteolysis in widely disparate biological systems (2). Ubiquitin is ligated to such target proteins via an isopeptide linkage by the action of a multi-enzymatic system consisting of E1 (Ub-activating), E2 (Ub-conjugating), and E3 (Ub-ligating) enzymes (1, 3). Protein ubiquitinylation is initiated by the formation of a high-energy thioester bond between Ub and an E1 in a reaction that requires ATP-hydrolysis. The activated Ub is then transferred to an E2. In some cases, E2 directly transfers Ub to target proteins, but the reaction often requires the participation of an E3. Finally, a poly-Ub chain is formed by linking the C-terminus of one Ub to a Lys residue within another Ub. The resultant poly-Ub chain acts as a degradation signal for proteolytic attack by the 26S proteasome. In this Ub-proteasome pathway, E3, the protein-Ub ligase, presumably plays the most important role in the selection of target proteins for degradation, because each distinct E3 binds the protein substrate with a degree of selectivity (1–3). Despite the importance of this step, the molecular basis of this Ub-ligating mechanism is largely unknown.

This situation has been true for the ubiquitination of the protein inhibitor IκB for the inducible transcriptional factor NF-κB that is involved in various biologically important processes, such as stress, inflammation, and immunity (4–6). Numerous accumulating evidence has revealed that activation of NF-κB is induced by signal-dependent degradation of IκB through the Ub pathway (7–9), indicating the importance of the IκB-Ub ligase (IκBα-E3) responsible for fine control of the level of IκB in cells. Although some reports suggest the existence of a specific E3 for IκBα (10, 11), no clear information has been available on this putative E3. Therefore, we attempted to identify an IκBα-Ub ligase from HeLa cells.

[1] To whom correspondence should be addressed. Fax: (+81)-3-3823-2237. E-mail: tanakak@rinshoken.or.jp.

0006-291X/99 $30.00

Here for the first time, the nature of such an IκB-E3 is described. It is a novel SCF-like complex containing Skp1, cullin-1, and two F-box/WD40-repeat proteins termed Hs-βTrCP1 and Hs-βTrCP2 (abbreviated simply βTrCP1 and βTrCP2 throughout this manuscript, unless otherwise specified). Hs-βTrCP1 is the human homologue of the *Drosophila* Slimb protein (12) and *Xenopus* βTrCP (13). Hs-βTrCP2 is a newly identified protein with great similarity to Hs-βTrCP1 which has been recently listed in a public database (Kazusa DNA Research Institute). These proteins are recruited into a complex which binds to phosphorylated IκBα produced by TNFα stimulation. The findings strongly indicate that IκBα-Ub ligase contains a complex similar to SCF (Skp1-cullin or Cdc53-[F-box] protein), a known protein-Ub ligase responsible for multiple cellular functions (14). The possible roles of this novel SCF-like complex for the signal-dependent ubiquitination of IκBα are discussed.

MATERIALS AND METHODS

Materials. Sources of materials are as follows: Ubiquitin (Ub)-aldehyde (MBL, Nagoya, Japan); MG115 (Z-Leu-Leu-nVal-H) and MG132 (Z-Leu-Leu-Leu-H) (Peptide Institute, Inc., Osaka, Japan); okadaic acid (OA) (Wako, Tokyo, Japan); human TNFα (Genzyme); bovine Ub, creatine phosphate, creatine kinase, and inorganic pyrophosphatase (Sigma); rabbit polyclonal antibodies against IκBα (amino acids 297–317, c-21), and mouse monoclonal anti-FLAG (M2) (Sigma). Polyclonal antibodies to human-Skp1 and human cullin-1 were raised in rabbits using recombinant proteins. Recombinant mouse E1 enzyme was purified from baculovirus-infected Sf9 insect cells (provided by K. Iwai, Kyoto University). Recombinant Ubch4 was produced in *E. coli* (provided by M. Nakao, Kumamoto University). IKK was purified from HeLa cell extracts, as described by Lee *et al.* (15).

Expression plasmids, in vitro translation, transfection and recombinant proteins. The pcDNA3FL-IκBα mammalian expression plasmid was constructed by inserting the cDNA encoding amino acids 2–317 of human IκBα into the *Bam*HI/*Not*I sites of pcDNA3FL (16). The Hs-βTrCP1 cDNA was amplified by PCR using a reported sequence (17). The Hs-βTrCP2 cDNA clone (KIAA0696) was generously donated by Kazusa DNA Research Institute (Chiba, Japan). The Hs-βTrCP1 and Hs-βTrCP2 cDNAs with a FLAG-sequence fused to the C-terminus were ligated into the pcDNA3.1 vector (Invitrogen). IκBα protein was produced and labeled with [^{35}S]methionine and [^{35}S]cystein using a wheat-germ lysate-based TNT transcription-translation coupled kit (Promega). Transient transfection for coimmunoprecipitation experiments was performed using the FuGENE 6 transfection reagent according to the manufacturer's instructions (Boehringer-Mannheim).

Cell culture and TNFα treatment. Human HeLa-S3 cells were maintained in MEM for suspension cells (GIBCO) supplemented with 5% fetal bovine serum, 2 mM glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin. After HeLa cells were pretreated with 50 μM MG132 for 45 min followed by 0.25 μM of okadaic acid (OA) for 15 min, human alpha-type tumor necrosis factor (TNFα) was added for 15 min at a final concentration of 300 units/ml.

Ubiquitinylation assay of IP-IκBα. HeLa cells (10^8 cells) treated with TNFα were suspended in 50 mM Tris–HCl (pH 7.4) buffer containing 1 mM EGTA, 2 mM DTT, 25 mM sodium β-glycerophosphate, 0.1 mM PMSF (phenylmethanesulfonyl phosphate), and 10 μg/ml each of leupeptin, aprotinin, and pepstatin A and then immediately lysed in a glass Dounce homogenizer. Lysates were clarified by low speed centrifugation (500 rpm) for 10 min, followed by further centrifugation at 15,000 rpm for 20 min. Immunoprecipitations (abbreviated IP) were performed with an anti-IκBα antibody and followed by washing four times with 20 mM Tris–HCl (pH 7.4) buffer containing 150 mM NaCl, 2 mM DTT, 25 mM sodium β-glycerophosphate, 0.1 mM PMSF, and 10 μg/ml each of leupeptin, aprotinin, and pepstatin A. The resulting immunoprecipitates were referred to as IP-pIκBα, which is the phosphorylated form.

The resulting slurries of IP-pIκBα were incubated in a reaction volume of 20 μl that included 2 μl of 10× ATP regenerating system containing 500 mM Tris (pH 7.6), 100 mM $MgCl_2$, 20 mM ATP, 5 mM DTT, 100 mM creatine phosphate, 35 U/ml of creatine kinase, and 6 U/ml inorganic pyrophosphatase, 0.5 μM okadaic acid, 5 μM MG115, 100 ng of recombinant mouse E1, 3 μg of $(His)_6$-Ubch4, and 1 mg/ml of bovine Ub, and 4–6 μl of IP-pIκBα. The reactions were incubated at 37°C for 3 h. After terminating the reaction by the addition of a sample buffer for SDS–PAGE, the reaction mixtures were separated by SDS–PAGE on a 10% gel followed by Western blotting with an anti-IκBα antibody.

Western analysis. For immunoblotting, the ubiquitination reaction mixtures and immunoprecipitates for various antibodies were separated by SDS–PAGE and transferred to a PVDF membrane. The membranes were probed with various antibodies against IκBα, FLAG peptide, Cul-1, and Skp1 and visualized with horseradish peroxidase-conjugated protein A (Bio-Rad) using the Enhanced Chemiluminescence Detection System (Amersham Pharmacia Biotech).

RESULTS

To clarify the biochemical nature of IκBα-E3, we thought that the mammalian homologue of *Drosophila* Slimb might participate in degradation of IκBα, in addition to β-catenin, because IκBα has two conserved Ser phosphorylation sites similar to β-catenin, which are necessary for the ubiquitination of both proteins (12, 18). Slimb is an F-box/WD40-repeat protein which negatively regulates the Wingless pathway and Hedgehog pathway. Based on its structural feature, Slimb was postulated to be a component of the SCF-like Ub ligase complex which would regulate the stability of Armadillo (*Drosophila* β-catenin). Taking advantages of the fact that the WD40-repeat domain of Slimb is highly conserved with a *Xenopus* homologue βTrCP (13), we screened a human placenta cDNA library (CLONTECH) with this sequence motif obtained from a human EST database. Two homologous but distinct cDNA clones provisionally named Hs-βTrCP1 and Hs-βTrCP2 were obtained by screening under low stringent hybridization conditions. βTrCP1 had identical nucleotide sequence to the probe used, while βTrCP2 had 95% identity. Hs-βTrCP1 turned out to be identical to h-βTrCP which was recently reported to interact with HIV-1 Vpu and Skp1 to trigger the destruction of CD4 in the endoplasmic reticulum (17). Furthermore, βTrCP2 was also recently listed as KIAA0696 in a database of Kazusa DNA Research Institute (Chiba, Japan). Figure 1 shows the alignment of the amino acid sequence of βTrCP1 with that of βTrCP2. A similarity between these proteins was observed throughout their

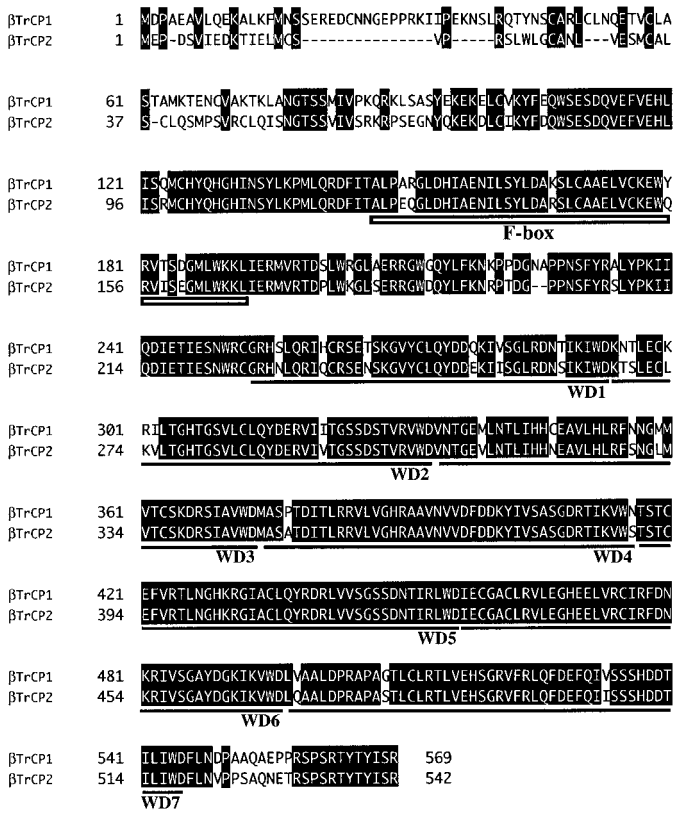

FIG. 1. Comparison of the protein sequences of Hs-βTrCP1 and Hs-βTrCP2. Gaps (shown by dashes) were inserted to achieve maximum sequence homologies. Identical amino acid residues are shown by black boxes. Amino acid residues are numbered at the left. The alignments were made by the CLUSTALW program (31). F-box and seven WD40 domains are indicated. Sequence data of Hs-βTrCP1 were taken from Margottin *et al.* (17) (accession number: Y14153), and those of Hs-βTrCP2 were listed in a public database from Kazusa DNA Research Institute (Chiba, Japan: KIAA0696).

entire sequences, displaying 78% amino acid identity. Intriguingly, the F-box motif and WD40-repeat domains were highly conserved between these proteins. Despite its high identity, it is of note that the *βTrCP1* and *βTrCP2* genes have been mapped on different chromosomes, 10q24-25 (Fujiwara *et al.*, submitted) and 5q33-34 (unigene database, NCBI), respectively.

To test whether βTrCP1 and βTrCP2 are involved in IκBα ubiquitination, plasmid DNA encoding FLAG-tagged human βTrCP1 and βTrCP2 were transfected into HeLa cells, and this interaction of the tagged βTrCP proteins with IκBα was examined by immunoprecipitation assay. Cells were treated with tumor necrosis factor-α (TNFα) or nothing, 36 h after transfection. These crude extracts were subjected to immunoprecipitation by either anti-FLAG or anti-IκBα antibody followed by Western blotting analysis with anti-FLAG, anti-IκBα, and anti-human Skp1 antibodies. With the anti-FLAG antibody, equal amounts of βTrCP1 or βTrCP2 from both TNFα-treated and untreated cell extracts were immunoprecipitated (Fig. 2). However, IκBα was only detected in βTrCP1 and βTrCP2 immunoprecipitates prepared from TNFα-treated HeLa cells, not in those from untreated cells. Conversely, an anti-IκBα antibody co-immunoprecipitated βTrCP1 and βTrCP2 from TNFα-treated HeLa cell extracts, but not from TNFα-untreated cells (upper panel). These results indicate that both βTrCP1 and βTrCP2 were specifically bound to phosphorylated IκBα in a signal-dependent fashion.

We next examined whether Skp1, a component of the SCF (Skp1/cullin or Cdc53/F-box protein) complex which binds to F-box motifs, also binds to phosphorylated IκBα. Immunoprecipitation of IκBα from HeLa cell extracts revealed that Skp1 is coimmunoprecipitated with pIκBα, but not with IκBα (Fig. 2, lower panel). Intriguingly, the endogenous Skp1 was co-immunoprecipitated with βTrCP1-FLAG, irrespective of TNFα stimulation, indicating that βTrCP1 is constitutively associated with Skp1 in both stimulated and unstimulated HeLa cells. These results imply that the βTrCP1 and Skp1 simultaneously bind to phosphorylated IκBα in a signal-dependent manner. Moreover, this association of βTrCP1 and Skp1 with IP-pIκBα was highly resistant to treatment with 1.0 M NaCl wash (data not shown), indicating that these two proteins are specifically and tightly associated with phosphorylated IκBα.

Until now, a typical SCF^{Cdc4} complex was known to consist of Skp1, Cdc53 (the mammalian cullin-1 (abbreviated Cul-1) homologue) and the F-box protein Cdc4. The complex is crucial in ubiquitinating the Cdk inhibitor Sic1 in the budding yeast (19, 20). In addition, it was found that Skp1 associates with Hs-Cul-1, but not with other human cullin family proteins (21). Therefore, we thought the binding of Cul-1 with the βTrCP1-Skp1 occurred in this complex. To ascertain this, each βTrCP1-FLAG and IκBα immunoprecipitate from TNFα-treated or untreated extracts was further probed with an anti-Cul-1 antibody. As shown in Fig. 3 (upper panel), Cul-1 was co-immunoprecipitated by either IκBα or βTrCP1-FLAG from TNFα treated extracts. In contrast, neither βTrCP1-FLAG nor IκBα coimmunoprecipitated Cul-1 from TNFα-untreated HeLa extracts. These findings clearly indicate that Cul-1 is not bound to βTrCP1 in the unstimulated HeLa cells, but it is recruited to phosphorylated IκBα when the βTrCP1-Skp1 complex is bound. Moreover, this association of Cul-1 with pIκBα was highly resistant to treatment with 1.0 M NaCl wash (Fig. 3, lower panel) as were βTrCP1 and Skp1 (Fig. 2), indicating that these three proteins are specifically and tightly associated with phosphorylated IκBα.

Finally it is of particular interest to examine whether βTrCP is functionally involved in the ubiquitination of IκBα. Recently, we found that a putative IκBα-Ub ligase (equivalent to IκBα-E3) is specifically associated with phosphorylated IκBα (abbreviated

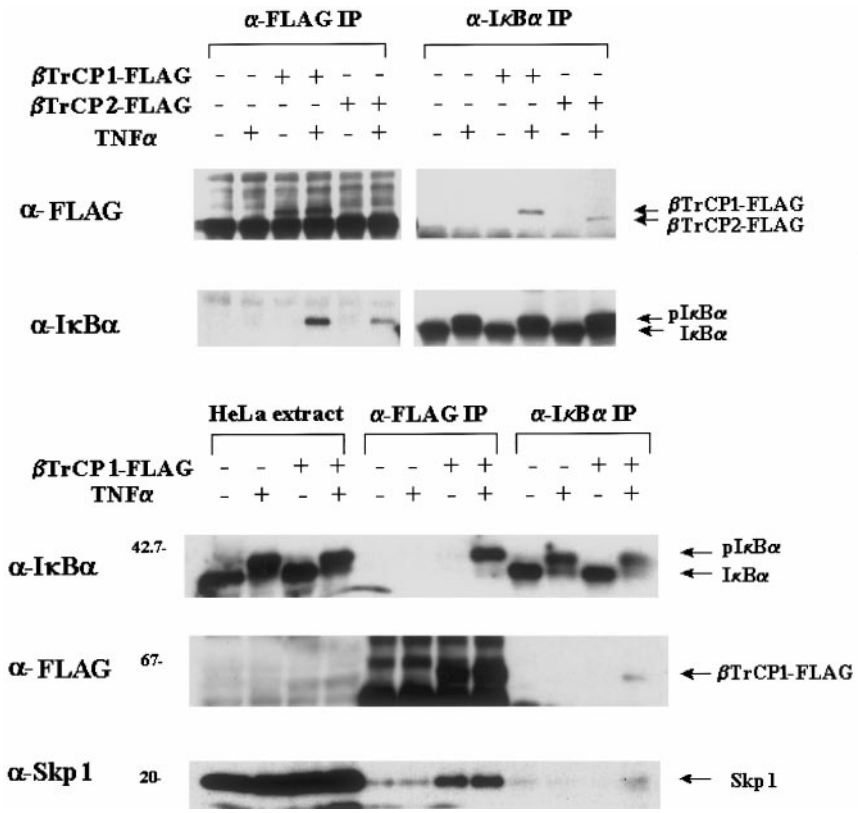

FIG. 2. Binding of βTrCP1, βTrCP2, and Skp1 to phosphorylated pIκBα. (Upper panel) Thirty-six hours after pcDNA3-βTrCP1-FLAG, pcDNA3-βTrCP2-FLAG or pcDNA3 (mock control) was transfected into HeLa cells and treated with either TNFα or nothing, crude extracts were prepared as described under Materials and Methods. After immunoprecipitation by anti-FLAG and anti-IκBα antibodies, the resulting immunoprecipitates and crude extracts were analyzed by Western blotting using anti-FLAG and anti-IκBα antibodies. (Lower panel) Experiments were the same as for (Upper panel), except that pcDNA3-βTrCP1-FLAG was transfected and Western blotting was carried out using anti-FLAG, anti-IκBα and anti-Skp1 antibodies.

pIκBα) produced by TNFα stimulation, but not with unphosphorylated IκBα (Suzuki *et al.*, submitted). When phosphorylated IκBα was prepared by immunoprecipitation using polyclonal antibodies from TNFα-treated HeLa cell extracts and then incubated with ubiquitin (Ub), ATP, recombinant mouse E1 and human E2 (Ubch4) enzymes, polyubiquitinated bands designated $(Ub)_n$-IκBα were detected by Western blot analysis with an anti-IκBα antibody (Fig. 4, lane 4). This reaction requires E1, E2, and Ub (lane 1), which suggests that a presumptive IκBα-Ub ligase (IκBα-E3) is tightly associated with IP-pIκBα *in vivo*. In contrast, these Ub-ligated bands were not detected for the non-phosphorylated form of IP-IκBα (data not shown).

Subsequently, to ascertain that βTrCP is functionally involved in the ubiquitination of IκBα, the effect of the over-expression of βTrCP1 or βTrCP2 protein on the ubiquitination activity of IP-pIκBα was examined. As shown in Fig. 4 (lanes 5 and 6), a poly-ubiquitination of IP-pIκBα prepared from TNFα-treated transfectants was markedly enhanced by the expression of βTrCP1 or βTrCP2. These augmented polyubiquitinated bands were not observed without the addition of Ub (lanes 2 and 3). These results strongly support that these two βTrCP proteins are functionally involved in the ubiquitination of IκBα.

DISCUSSION

The selective degradation of IκB plays a pivotal role in the NF-κB signaling pathway because IκB antagonizes signal transduction for numerous stimulators in a wide-variety of cells. Notably, two important discoveries have broadened the understanding of the molecular mechanism of IκB destruction. First was the discovery of the IκB kinase (IKK) complex, which catalyzes the phosphorylation of IκB (22–24). IKK was found to be a surprisingly large, multi-protein complex with a molecular mass of 700–900 kDa. Although much has been learned about its function, the details of its functions have not yet been conclusively ascertained. The second important finding was that phosphorylated IκB is degraded by the ubiquitination pathway, which is a necessary process for the transduction of NF-κB signaling (4, 14, 15, 25–27). However, little is known to date of an IκB-Ub ligase (IκB-E3), which

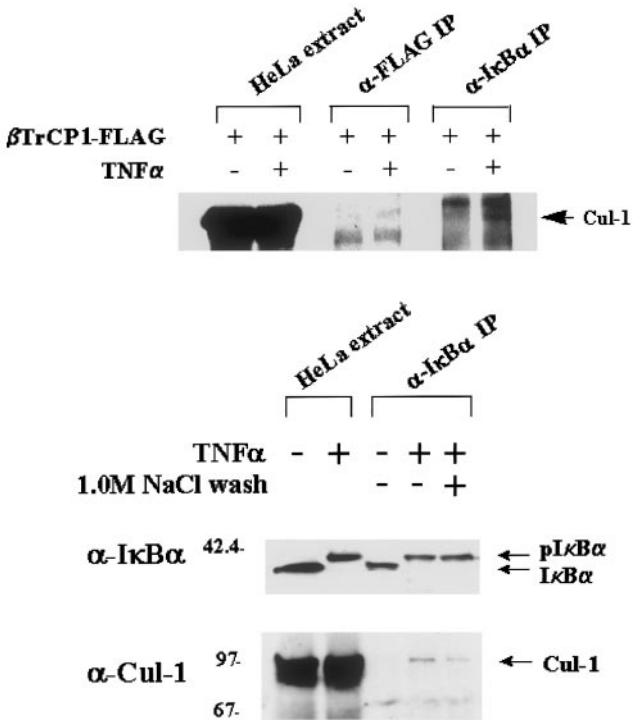

FIG. 3. Interaction of Hs-Cul-1 with βTrCP1 and pIκBα. After HeLa cells were transfected with either pcDNA3-βTrCP1-FLAG (upper panel) or nothing (lower panel), TNFα treatment, preparation of crude extracts, and immunoprecipitation (IP) by anti-FLAG or anti-IκBα antibodies were carried out as in Fig. 2, except that IP-pIκBα in the lower panel was washed with 1.0 M NaCl. Western analysis was carried out using an anti-Hs-Cul-1 antibody.

should be the most important enzyme in the ubiquitination of IκB.

In the present study, we found such a presumptive IκBα-ligating enzyme. This enzyme is thought to be an SCF-like complex. SCF is a multifunctional Ub-protein ligase E3 complex that plays a critical role in Ub-mediated proteolysis (14). In the budding yeast *S. cerevisiae,* Cdc4 is an F-box/WD40 repeat protein that functions as a component of a specific SCF consisting of a Skp1-Cdc53-Cdc4 complex responsible for the recognition and ubiquitinylation of the phosphorylated Cdk inhibitor, Sic1p (19, 20). We provided similar evidence that βTrCP, which possesses an F-box/WD40-repeat protein related to Cdc4, is a component of IκBα-E3 that specifically associates with phosphorylated IκBα, but not with the unphosphorylated form. Interestingly, here we reported two homologous βTrCP proteins termed βTrCP1 and βTrCP2 that were recruited to associate with phosphorylated IκBα, but not with the unphosphorylated form (Fig. 2). Indeed, transfection of these two cDNAs in HeLa cells resulted in dramatic augmentation of the *in vitro* poly-ubiquitination of pIκBα (Fig. 4), implying that these two βTrCP proteins are functionally involved in the ubiquitin-mediated degradation of IκBα. We further provided direct evidence that Skp1 forms a complex with βTrCP1 in the HeLa cells, irrespective of TNFα stimulation. This complex then binds to phosphorylated IκBα in a signal-dependent manner, suggesting that βTrCP1 and Skp1 play essential roles in the function of the IκBα-Ub ligase complex. In contrast, Cul-1 was not associated with the βTrCP1-Skp1 complex in unstimulated HeLa cells, but it was also recruited to phosphorylated IκBα in response to TNFα signaling (Fig. 3). Thus, it becomes clear that these βTrCP, Skp1, and Cul-1 are tightly assembled into a complex which binds to phosphorylated IκBα upon TNFα stimulation. These data strongly indicate that IκBα-E3 is equivalent to an SCF-like complex consisting of βTrCP, Skp1, and Cul-1, although the possibility that other factor(s) is also recruited into the IκBα-E3 complex cannot be ruled out.

During the preparation of this manuscript, Yaron *et al.* (11) reported that a βTrCP-like protein was the receptor component of an IκBα-Ub ligase complex, although they could not identify Skp1 and Cul-1. The βTrCP-like protein seems to correspond to βTrCP1, although it has the insertion of approximately 30 amino acid residues in the N-terminal region in comparison with the primary structure of human βTrCP reported by Margottin *et al.* (17), implying the presence of variant forms for βTrCP1. Yaron *et al.* (11) has chemically sequenced three peptide fragments of βTrCP associated with phosphorylated IκBα, though these sequences were common in both βTrCP1 and βTrCP2. Indeed, in our present work, we found that

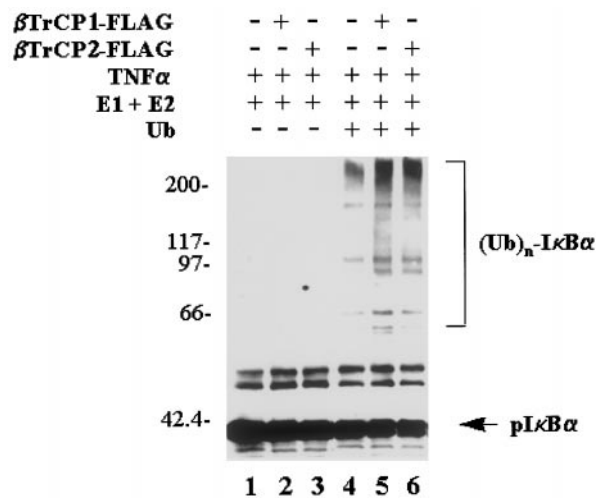

FIG. 4. Augmentation of poly-ubiquitinylation with IP-pIκBα by transfection of βTrCP1 and βTrCP2 cDNAs. Transfection of pcDNA3-βTrCP1-FLAG, pcDNA3-βTrCP2-FLAG or pcDNA3 and TNFα treatment were carried out as in Fig. 2. Ubiquitination assay by IP-pIκBα prepared from these transfectants was performed as described under Materials and Methods. IP-IκBα was incubated with ATP, E1, and $(His)_6$-Ubch4 (E2) in the presence (+) or absence (−) of Ub. Multiple ubiquitinated IκBα bands with higher molecular masses were detected by Western blot analysis and are designated $(Ub)_n$-IκBα.

both βTrCP1 and βTrCP2 were recruited to associate with phosphorylated IκBα in a signal-dependent fashion, suggesting that two homologous βTrCP proteins may play important roles in the ubiquitinylation of IκBα. Further study is required to elucidate their functions.

Polyubiquitination of β-catenin and IκB appears to be operated by the same mechanism, requiring phosphorylation prior to ubiquitination. However, β-catenin is phosphorylated by glycogen synthase kinase-3β (GSK-3β), whose binding to β-catenin is stabilized by the gene product of adenomatous polyposis coli (APC) and axin (28). Interestingly, the primary structure of the phosphorylation sites of β-catenin by GSK-3β remarkably resembles that of IκB by IKK, implying that the same type of Ub-ligase may catalyze the polyubiquitinylation of both β-catenin and IκB. This hypothesis is quite attractive, but speculative. In addition, it is plausible that many other cell-cycle mediators, such as various cylins B, D1, and E and Cdk (*c*yclin-*d*ependent protein *k*inase 1) inhibitors, such as Sic1, Rum1, Far1, p21, and p27, are also degraded by the Ub-proteasome pathway. Interestingly, most such cell-cycle factors are modified by phosphorylation prior to ubiquitinylation and degradation as is the case for IκB (29). Remarkably, of these, Sic1 and Rum1 are degraded by a Ub pathway mediated by SCF E3 ligase (19, 20, 30). Therefore, our understanding of the mechanism of IκB ubiquitination by an SCF-like complex is important not only for greater understanding of the NF-κB signaling pathway, but also in opening new insights into the larger biological significance of the Ub-proteasome pathway in development, disease and death.

ACKNOWLEDGMENTS

The authors thank Steven E. Johnson for editing the manuscript. We are also grateful to all other members of MML in Yamanouchi Pharmaceutical Co., Ltd., for encouragement and support. This work was supported in part by grants from the program Grants-in-Aid for Scientific Research on Priority Areas (Intracellular Proteolysis) from the Ministry of Education, Science, Sports, and Culture of Japan and the Human Frontier Science Promotion Organization (K.T.).

REFERENCES

1. Hershko, A., and Ciechanover, A. (1998) *Annu. Rev. Biochem.* **67,** 425–479.
2. Ciechanover, A. (1998) *EMBO J.* **17,** 7151–7160.
3. Hochstrasser, M. (1996) *Annu. Rev. Genet.* **30,** 405–439.
4. Baeuerle, P. A., and Baltimore, D. (1996) *Cell* **87,** 13–20.
5. Baldwin, A. S. (1996) *Annu. Rev. Immunol.* **14,** 649–681.
6. May, M. J., and Ghosh, S. (1998) *Immunol. Today* **19,** 80–88.
7. Finco, T. S., and Baldwin, A. S. (1995) *Immunity* **3,** 253–272.
8. Traenckner, E. B.-M., Wilk, S., and Baeuerle, P. A. (1994) *EMBO J.* **13,** 5433–5441.
9. Chen, Z. J., Parent, L., and Maniatis, T. (1996) *Cell* **84,** 853–862.
10. Yaron, A., Gonen, H., Alkalay, I., Hatzubai, A., Jung, S., Beyth, S., Mercurio, F., Manning, A. M., Ciechanover, A., and Ben-Neriah, Y. (1997) *EMBO J.* **16,** 5486–6494.
11. Yaron, A., Hatzubai, A., Davis, M., Lavon, I., Amit, S., Manning, A. M., Anderson, J. S., Mann, M., Mercurio, F., and Ben-Neriah, Y. (1998) *Nature* **396,** 590–594.
12. Jiang, J., and Struhl, G. (1998) *Nature* **391,** 493–496.
13. Marikawa, Y., and Elinson, R. P. (1998) *Mech. Dev.* **77,** 75–80.
14. Patton, E. E., Willems, A. R., and Tyers, M. (1998) *Trends Genet.* **14,** 236–243.
15. Lee, F. S., Hagler, J., Chen, Z. J., and Maniatis, T. (1997) *Cell* **88,** 213–222.
16. Takeuchi, M., Rothe, M., and Goeddel, D. V. (1996) *J. Biol. Chem.* **271,** 19935–19942.
17. Margottin, F., Bour, S. P., Durand, H., Selig, L., Benichou, S., Richard, V., Thomas. D., Strebel, K., and Benarous, R. (1998) *Mol. Cell* **1,** 565–574.
18. Aberle, H., Bauer, A., Stappert, J., Kispert, A., and Kemler, R. (1997) *EMBO J.* **16,** 3797–3804.
19. Skowyra, D., Craig, K. L., Tyers, M., Elledge, S. J., and Harper, J. W. (1997) *Cell* **91,** 209–219.
20. Feldman, R. M., Correll C. C., Kaplan, K. B., and Deshaies, R. J. (1997) *Cell* **91,** 221–230.
21. Michel, J. J., and Xiong, Y. (1998) *Cell Growth Differ.* **9,** 435–449.
22. Maniatis, T. (1997) *Science* **278,** 818–819.
23. Israël, A. (1997) *Nature* **388,** 519–521.
24. Scheiderelt, C. (1998) *Nature* **395,** 225–226.
25. Scherer, D. C., Brockman, J. A., Chen, Z., Maniatis, T., and Ballard, D. W. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 11259–11263.
26. Baldi, L., Brown, K., Franzoso, G., and Siebenlist, U. (1996) *J. Biol. Chem.* **271,** 376–379.
27. Rodriguez, M. S., Wright, J., Thompson, J., Thomas, D., Baleux, F., Virelizier, J. L., Hay, R. T., and Arenzana-Seisdedos, F. (1996) *Oncogene* **12,** 2425–2435.
28. Hart, M. J., de los Santos, R., Albert, I. N., Rubinfeld, B., and Polakis, P. (1998) *Curr. Biol.* **8,** 573–581.
29. Deshaies, R. J. (1997) *Curr. Opin. Gen. Dev.* **7,** 7–16.
30. Kominami, K., and Toda, T. (1997) *Genes Dev.* **11,** 1548–1560.
31. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.* **22,** 4673–4680.