Common Pathway for the Ubiquitination of IκBα, IκBβ, and IκBε Mediated by the F-Box Protein FWD1*

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FWD1 (the mouse homolog of Drosophila Slimb and Xenopus βTrCP, a member of the F-box- and WD40 repeat-containing family of proteins, and a component of the SCF ubiquitin ligase complex) was recently shown to interact with IκBα and thereby to promote its ubiquitination and degradation. This protein has now been shown also to bind to IκBβ and IκBε as well as to induce their ubiquitination and proteolysis. FWD1 was shown to recognize the conserved DSGΨXS motif (where Ψ represents the hydrophobic residue) present in the NH2-terminal regions of these three IκB proteins only when the component serine residues are phosphorylated. However, in contrast to IκBα and IκBβ, the recognition site in IκBε for FWD1 is not restricted to the DSGΨXS motif; FWD1 also interacts with other sites in the NH2-terminal region of IκBε. Substitution of the critical serine residues in the NH2-terminal regions of IκBα, IκBβ, and IκBε with alanines also markedly reduced the extent of FWD1-mediated ubiquitination of these proteins and increased their stability. These data indicate that the three IκB proteins, despite their substantial structural and functional differences, all undergo ubiquitination mediated by the SCF (FWD1 complex. FWD1 may thus play an important role in NF-κB signal transduction through regulation of the stability of multiple IκB proteins.

The transcription factor nuclear factor-κB (NF-κB)1 plays a central role in the activation of many genes that are important in inflammatory and immune responses as well as in the response to cellular stress (1–4). NF-κB consists of homo- and heterodimeric complexes of members of the Rel family of proteins. To date, five Rel proteins have been identified in mammals as follows: p50, p52, p65 (RelA), c-Rel, and Rel-B, the first two of which are synthesized as p105 and p100 precursors, respectively (4–6). These proteins bind specifically to κB motifs located in the promoters and enhancers of target genes, resulting in transcriptional activation. Furthermore, they all share an ~300-amino acid region of homology, known as the Rel homology domain, that is responsible for DNA binding, dimerization, and interaction with inhibitory proteins of the IκB family.

The IκB family of inhibitory proteins includes IκBα, IκBβ, IκBε, IκBγ, and Bcl-3 in higher vertebrates (Fig. 1A), all of which contain multiple regions of homology known as ankyrin-repeat motifs (4). Such repeats mediate protein-protein interactions, and the specific interaction between ankyrin repeats of IκB proteins and the Rel homology domain of Rel proteins appears to be an important and evolutionarily conserved feature of the regulation of NF-κB (7). The number of ankyrin repeats varies among the different IκB proteins and appears to influence the specificity with which IκB pairs with a Rel dimer. The Rel precursor proteins p100 and p105 also contain ankyrin repeats and are sometimes included in the IκB family.

IκBα, the best characterized member of the IκB family, is a 37-kDa protein with a tripartite structural organization that is also apparent in IκBβ and which consists of (i) an NH2-terminal domain that is phosphorylated in response to extracellular signals, (ii) a central ankyrin-repeat domain, and (iii) a COOH-terminal PEST domain that is important in the basal turnover of the protein (6, 8, 9). The defining functional characteristic of IκBα is its ability to confer rapid but transient induction of NF-κB activity as a result of its participation in an autoregulatory feedback loop. Activation of NF-κB leads to up-regulation of transcription of the IκBα gene, and the consequent increase in the amount of IκBα serves to shut off the activation signal (10–15).

IκBβ is a 45-kDa protein and, like IκBα, binds p50-p65 and p50-p-c-Rel complexes (16, 17). Functionally, IκBβ and IκBα differ with regard both to the nature of the incoming signal as well as to the timing of the onset of and the duration of the response (18). Both IκBα and IκBβ are rapidly degraded after exposure of cells to an appropriate stimulus; however, whereas transcription of the IκBα gene is induced as a result of NF-κB activation that of the IκBβ gene is not (16). Thus, the abundance of IκBβ remains low until the NF-κB-activating signal is attenuated.

IκBε is degraded with slower kinetics than are IκBα and IκBβ (17, 19). Unlike IκBα and IκBβ, IκBε contains a cluster of serine residues at the NH2 terminus and lacks a PEST domain at the COOH terminus (Fig. 1A), suggesting that these differences in structure may underlie the differences in function. The NH2-terminal region of IκBε is rich in Pro, Glu, Asp, Ser, and Thr residues (61 out of 118 residues; 51.7%) and resembles a PEST sequence (17). IκBε does not interact with either p50 or p52; instead, it associates exclusively with p65-p65 and p65-c-Rel complexes and therefore likely regulates the transcription of genes, such as that for interleukin-8, whose promoters bind preferentially to p65 and c-Rel complexes (19, 20).

The dimeric NF-κB complex is normally sequestered in an...
The ubiquitin-proteasome pathway of protein degradation is an important mechanism by which the abundance of specific cellular proteins is regulated (34–36). The formation of ubiquitin-protein conjugates is mediated by three enzymes that participate in a series of ubiquitin transfer reactions: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin ligase (E3). The specificity of protein ubiquitination often derives from the E3 ubiquitin ligases. Proteins that are polyubiquitinated by these enzymes are subjected to degradation by the 26 S proteasome (36). Recent genetic and biochemical studies in yeast have identified a class of E3 ligases, termed SCF complexes, that are required for degradation of cyclins and their inhibitors as well as for that of an increasing number of other proteins (37–39). SCF complexes consist of the invariant components Skp1 and Cdc53 (Cullin or Cul1) as well as a variable component known as an F-box protein, which binds to Skp1 through the F-box motif. F-box proteins serve as receptors for the target protein, which is usually phosphorylated.

We and others (40–48) recently showed that FW1D, the mouse homolog of Drosophila Slimb and Xenopus βTrCP and a member of the F-box and WD40 repeat family of proteins, is specifically associated with IκBa and β-catenin only when the latter is phosphorylated at the serine residues in the DSGVXS motif. FW1D also interacts with the Skp1-Cul1 complex through its F-box domain, thereby forming an SCF complex, SCF\textsuperscript{FW1D}. Thus, the phosphorylation of IκBa results in the recruitment of FW1D, which links IκBa to the ubiquitination machinery. SCF\textsuperscript{FW1D} therefore plays an important role in transcriptional regulation by NF-κB as a result of its control of IκBa protein stability.

We have now investigated whether FW1D contributes to ubiquitin-mediated proteolysis of IκBa family members other than IκBa. Our data show that IκBβ and IκBe are also ubiquitinated and that FW1D mediates the ubiquitination of these proteins in response to their phosphorylation by the IKK complex, thereby triggering their degradation.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—**293T cells were grown at 37°C and under an atmosphere of 5% CO\textsubscript{2} in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10% (v/v) fetal bovine serum (Life Technologies, Inc.).

**Construction of Expression Plasmids and Mutagenesis—**Molecular cloning of the FW1D and FW1D\textsubscript{ΔF} cDNAs was described previously (40, 41). Complementary DNAs encoding FW1D, FW1D\textsubscript{ΔF}, and FW1D\textsubscript{ΔF}, each protein tagged at the NH\textsubscript{2} terminus with the FLAG or Myc epitope, were generated with the use of the polymerase chain reaction, as performed with the high fidelity thermostable DNA polymerase KOD (Toyobo, Tokyo, Japan); they were then sequenced and subcloned into pcDNA3 (Invitrogen, Carlsbad, CA). FW1D\textsubscript{ΔF} contains residues 1–140 of FW1D fused to residues 194–569. IκBa, IκBβ, IκBe, and IκKB cDNAs were kindly provided by H. Nakano (27). The IκB cDNAs were subcloned into pcDNA3 with the FLAG or Myc epitope tag, and the cDNA encoding FLAG- or Myc-tagged IKKβ was cloned into the TA Cloning System (Invitrogen).

**Transfection, Immunoprecipitation, and Immunoblot Analysis—**293T cells were transfected by the calcium phosphate method or with the use of the LipofectAMINE reagent (Life Technologies, Inc.). After 48 h, the cells were lysed by a solution containing 50 mM Tris-HCl (pH 7.6), 300 mM NaCl, 0.5% Triton X-100 (v/v), aprotinin (10 μg/ml), leupeptin (10 μg/ml), 1 mM iodoacetamide, 1 mM phenylmethylsulfonyl fluoride, 0.4 mM Na\textsubscript{3}VO\textsubscript{4}, 0.4 mM EDTA, 10 mM NaF, and 10 mM sodium pyrophosphate. The cell lysates were pretreated with 2 μl of protein G-Sepharose beads (Amersham Pharmacia Biotech) for 1 h at 4°C and were then incubated with 5 μg of the appropriate antibodies and protein G-Sepharose beads for 4 h at 4°C. The resulting immunoprecipitates were then washed thoroughly four times with ice-cold lysis buffer, fractionated by SDS-polyacrylamide gel electrophoresis (PAGE), and subjected to immunoblot analysis with antibodies (1 μg/ml) to Myc (9E10; Roche Diagnostics, Tokyo, Japan), to the FLAG epitope (M5, Sigma), or to ubiquitin (1B3, MBL, Nagoya, Japan).
FWD1 Mediates Ubiquitination of IκBβ and IκBe

RESULTS

**FWD1-induced Ubiquitination of IκBα, IκBβ, and IκBe**—We and others (40, 42–44, 46, 47) have recently shown that the FWD1 component of the ubiquitin ligase complex SCF<sup>WD1</sup> serves as an intracellular receptor for Iκβ that has been phosphorylated at the DSG<sup>XS</sup> motif by IKK. We therefore investigated whether FWD1 also associates with IκBβ and IκBe through their phosphorylated DSG<sup>XS</sup> motifs. Expression plasmids encoding FLAG-tagged IκBα, IκBβ, IκBe, or their SA mutants, in which serine was replaced with alanine at positions 32 and 36 (S32A/S36A), 19 and 23 (S19A/S23A), or 18 and 22 (S18A/S22A), respectively, were introduced into 293T cells with or without plasmids encoding Myc-tagged IKKβ, AU1-tagged MEKK1, and Myc-FWD1 together. Immunoprecipitation assays revealed that Myc-tagged FWD1 was present in the IκBα, IκBβ, and IκBe immunoprecipitates prepared with antibodies to FLAG (Fig. 2). The SA mutants of IκBα and IκBβ did not interact with FWD1, indicating that FWD1 recognizes the serine-phosphorylated DSG<sup>XS</sup> motif. In contrast, association of the SA mutant of IκBe with FWD1 was apparent. The observed pattern of ubiquitination of the IκB proteins was consistent with the pattern of FWD1 binding. FWD1 markedly increased the ubiquitination of wild-type IκBα and IκBβ but not that of the corresponding SA mutants. In contrast, both wild-type IκBe and its SA mutant were ubiquitinated in the presence of FWD1, although the extent of ubiquitination of the SA mutant was less than that apparent with the wild-type protein. These results indicate that FWD1 interacts with all three IκB proteins and thereby promotes their ubiquitination, although the mode of binding appears to differ between IκBα and IκBβ on the one hand and IκBe on the other, probably as a result of the corresponding structural differences between these proteins: IκBe contains additional serine residues present within SXXXXS motifs in the NH<sub>2</sub>-terminal region and lacks a COOH-terminal PEST domain (Fig. 1).

**Phosphorylation-induced Ubiquitination of IκBβ Mediated by FWD1**—To investigate further the role of FWD1 in IκBβ ubiquitination, we examined whether IKK activity is required for this reaction. Expression of IKKβ alone with IκBβ in 293T cells resulted in a small increase in the extent of IκBβ ubiquitination (Fig. 3), which was likely mediated by endogenous FWD1. However, expression of both IKKβ and FWD1 together with IκBβ induced a marked increase in IκBβ ubiquitination. The interaction of IκBβ with FWD1 was observed only in the presence of exogenous IKKβ. Another mammalian F-box and WD40 repeat protein, FWD2 (or MD6), neither interacted with IκBβ, even in the presence of IKKβ, nor increased the extent of its ubiquitination, confirming that the interaction between FWD1 and IκBβ is specific. Furthermore, FWD1 did not bind to the S19A/S23A mutant of IκBβ in the presence of IKKβ. These results suggest that FWD1-induced ubiquitination requires prior phosphorylation of IκBβ on Ser-19 and Ser-23 by the IKK complex.

**Requirement of the F-Box Domain in FWD1 for IκBβ Ubiquitination**—We previously showed that FWD1 links IκBα to
the Skp1-Cul1 complex as a result of the interaction of Skp1 with the F-box motif of FWD1. We therefore examined whether the F-box domain of FWD1 is also essential for the ubiquitination of IκBβ. The FWD1(ΔF) mutant, which lacks the F-box domain (amino acids 148 to 192), did not induce ubiquitination of IκBβ in the presence of IκKβ, although FWD1(ΔF) did interact with IκBβ (Fig. 4A). A pulse-chase experiment indicated that expression of FWD1 increased the rate of degradation of IκBβ relative to that apparent in cells expressing FWD2 (Fig. 4B). The FWD1(ΔF) mutant had no such effect, consistent with its inability to induce ubiquitination of IκBβ. Together, these observations suggest that FWD1, acting as a component of the SCF<sub>FWD1</sub> complex, functions as an intracellular receptor for IκBβ and thereby promotes the ubiquitination and degradation of this protein.

**FWD1-induced Ubiquitination of IκBe**—We have shown that FWD1 promotes the ubiquitination of IκBe (Fig. 2). However, unlike IκBe and IκBβ, the recognition motif for FWD1 in IκBe was not restricted to the phosphorylated DSG<sub>VS</sub> sequence because the S18A/S22A mutant of IκBe was also ubiquitinated, although to a reduced extent compared with that apparent with the wild-type protein. We therefore investigated whether phosphoserine residues located within other SXXXS motifs in the NH<sub>2</sub>-terminal region of IκBe contribute to FWD1-mediated ubiquitination of this protein. FWD1 bound to IκBe and elicited marked ubiquitination in the absence of exogenous IκKβ (Fig. 5A), whereas the ubiquitination of IκBe and IκBβ requires exogenous IκK activity. Furthermore, an IκBe mutant (SA-all) in which all nine serine residues within SXXXS motifs were replaced with alanines also associated with FWD1 and was ubiquitinated, although to a reduced extent relative to that observed with the wild-type protein (Fig. 5A). An IκBe mutant (∆N) that lacked the entire NH<sub>2</sub>-terminal domain (residues 1–118) neither bound to FWD1 nor was ubiquitinated. Thus, the susceptibility to ubiquitination of the various IκBe proteins examined decreases in the rank order wild-type > S18A/S22A > SA-all > ∆N. These results suggest that FWD1 interacts predominantly with Ser-18 and Ser-22 within the DSG<sub>VS</sub> motif of IκBe but is also able to bind to other serine residues in the NH<sub>2</sub>-terminal region of the protein.

With regard to the observation that FWD1-mediated ubiquitination of IκBe did not require exogenous IκK, it was possible that FWD1 interacted with IκBe in an IκK-independent manner. Alternatively, the small amount of endogenous IκK might have been sufficient to promote the FWD1-IκBe interaction. Immunoblot analysis indeed revealed the presence of endogenous IκKβ in 293T cells (data not shown). We investigated these two possibilities by introducing a kinase-negative mutant of IκKβ (K44M) into 293T cells to inhibit the endogenous IκK activity. This mutant markedly inhibited the interaction between FWD1 and IκBe as well as FWD1-induced ubiquitination of IκBe in a dose-dependent manner (Fig. 5B), suggesting that endogenous IκK is required for both these events. Thus, whereas signal-induced activation of the IκK complex is required for the proteolysis of IκBa and IκBβ, IκBe appears to be constitutively degraded as a result of the low level of endogenous IκK activity.

**FWD1-induced Degradation of IκBa, IκBβ, and IκBe**—To investigate further whether the phosphorylation-induced interaction with FWD1 promotes the degradation of IκBa, IκBβ, and IκBe, we performed pulse-chase experiments (Fig. 6). In the presence of FWD1 and IκKβ, both IκBa and IκBβ were rapidly degraded, although the kinetics of IκBβ degradation were slightly slower than those of IκBa degradation. The rates of degradation of the IκBa(S32A/S36A) and IκBβ(S19A/S23A) mutants were markedly reduced relative to those of the corresponding wild-type proteins, indicating that FWD1 promotes degradation of both IκBa and IκBβ after specific phosphorylation of the DSG<sub>VS</sub> motif. The half-life of IκBe was longer than those of IκBa and IκBβ, as previously demonstrated (17). The IκBe(SA-all) mutant was more stable than was wild-type IκBe, consistent with our observation that the extent of ubiquitination of the mutant was markedly reduced compared with that of the wild-type protein (Fig. 5A). Thus, phosphorylation of serine residues present within SXXXS motifs in its NH<sub>2</sub>-terminal region appears to influence the stability of IκBe.

**DISCUSSION**

We and others (40–48) previously showed that FWD1 mediates the ubiquitination of IκBa and β-catenin by functioning as an intracellular receptor that links these substrates to the core complex of the SCF<sub>E3</sub> ubiquitin ligase. Human FWD1 was also shown to interact with the Vpu protein of human immunodeficiency virus-type 1 (49). IκBa, IκBβ, IκBe, β-catenin, and Vpu all share a DSG<sub>VS</sub> motif (Fig. 1B), the two serines in which undergo signal-induced phosphorylation. This shared property suggested that FWD1 might recognize the phosphorylated DSG<sub>VS</sub> motif in each of these proteins, as we showed was the case for IκBa. The phosphorylated DSG<sub>VS</sub> motif thus might constitute a signal for FWD1-mediated ubiquitination. In the
The NH₂-terminal region of IκBα contains a putative PEST domain which may contribute to its degradation by the proteasome. Indeed, our data indicate that the NH₂-terminal region of all three proteins; however, IκBα contains four additional S motifs clustered in this region (Fig. 1C). Although FWD1 interacts with IκBα, IκBβ, and IκBε, the mode of interaction with IκBα and IκBβ appears to differ from that for IκBε. Thus, mutation of the two serines in the DSGΨXS motif of IκBα and IκBβ to alanines prevented both the interaction of these proteins with FWD1 and their FWD1-mediated ubiquitination as well as increased their stability. These data indicate the absolute requirement for the DSGΨXS motif in the interaction of IκBα and IκBβ with FWD1. In contrast, mutation of the two serines in the DSGΨXS motif of IκBε did not prevent its binding to FWD1. Furthermore, the IκBε(SA-all) mutant, in which the serines in all five S motifs were replaced by alanines, also interacted with FWD1, although the extent of its ubiquitination was substantially reduced compared with that of the wild-type protein. Hence, FWD1 appears to recognize motifs other than S motifs specifically on Ser-18 and Ser-22 in trans-association with FWD1 and its ubiquitination suggests that although FWD1 binding to and efficient ubiquitination of these three IκB proteins, FLAG-IKKβ (wild-type, wt) IκBα, IκBβ and FLAG-FWD1, in the absence or presence of a kinase-negative mutant of IKKβ (K44M) at increasing concentrations, as indicated. Cell lysates were subjected to immunoprecipitation with antibodies to Myc, to FLAG, or to ubiquitin as indicated. A portion of the cell lysates corresponding to 10% of the input for immunoprecipitation was also subjected to immunoblot analysis with antibodies to FLAG in order to indicate the levels of expression of p27 and FWD1. B, effect of a kinase-negative IKKβ mutant (K44M) on the binding of IκBα to FWD1 and its ubiquitination. 293T cells were transfected with expression plasmids encoding Myc-IκBα and FLAG-FWD1, in the absence or presence of a vector encoding IKKβ(K44M) at increasing concentrations, as indicated. Cell lysates were subjected to immunoprecipitation with antibodies to Myc, and the resulting precipitates were subjected to immunoblot analysis with antibodies to Myc, to FLAG, or to ubiquitin. A portion of the cell lysates corresponding to 10% of the input for immunoprecipitation was subjected to immunoblot analysis with antibodies to FLAG to indicate the levels of expression of IKKβ(K44M) and FWD1.

In the present study, we therefore investigated whether FWD1 also mediates the ubiquitination of IκBβ and IκBε. Indeed, our data show that FWD1 also serves to link these two proteins to the core complex of the SCF ubiquitin ligase in a phosphorylation-dependent manner.

The structural organization of IκBε differs from that of IκBα and IκBβ (4). Whereas IκBα and IκBβ contain a PEST domain in the COOH-terminal region, IκBε contains a putative PEST domain in the NH₂-terminal region, upstream of the ankyrin repeats (Fig. 1A) (17). The DSGΨXS motif is present in the NH₂-terminal regions of all three proteins; however, IκBε also contains four additional SXXX motifs clustered in this region (Fig. 1C). Although FWD1 interacts with IκBα, IκBβ, and IκBε, the interaction of these proteins with FWD1 and their FWD1-mediated ubiquitination as well as increased their stability. These data indicate the absolute requirement for the DSGΨXS motif in the interaction of IκBα and IκBβ with FWD1. In contrast, mutation of the two serines in the DSGΨXS motif of IκBε did not prevent its binding to FWD1. Furthermore, the IκBε(SA-all) mutant, in which the serines in all five SXXX motifs were replaced by alanines, also interacted with FWD1, although the extent of its ubiquitination was substantially reduced compared with that of the wild-type protein. Hence, FWD1 appears to recognize motifs other than SXXX in IκBε. Together, our data indicate that FWD1 is important for the ubiquitination of these three IκB proteins, although the manner of association appears to differ between IκBα and IκBβ on the one hand and IκBε on the other hand.

Another difference between IκBα and IκBβ versus IκBε is that although FWD1 binding to and efficient ubiquitination of IκBα and IκBβ in 293T cells required introduction of both FWD1 and IKKβ, IκBε interacted with FWD1 and underwent ubiquitination in the presence of exogenous FWD1 alone (without introducing exogenous IKKβ). Our observation that a kinase-negative mutant of IKKβ (K44M) inhibited both the interaction of IκBε with FWD1 and its ubiquitination suggests that endogenous IKK activity was necessary and sufficient for these reactions to proceed. However, we also showed that IKKβ phosphorylated IκBε specifically on Ser-18 and Ser-22 in transfected 293T cells (data not shown). This result appears inconsistent with the observation that the IκBε(S18A/S22A) mutant retained the ability to undergo ubiquitination, but it might be explained by IKKβ phosphorylation of other kinases or modu-
lators that promote the interaction of FWD1 with IkB.

Although IkBa, IkB, and IkBe all appear to undergo FWD1-mediated ubiquitination, and at least IkBa and IkB seem to be phosphorylated in a similar manner, the three proteins exhibited differential stabilities. It is possible that factors other than phosphorylation of the DSGVXS motif affect the interaction of FWD1 with the IkB proteins. Simeonidis et al. (7) showed that the structural differences in the NH2-terminal regions of IkBa, IkB, and IkBe may contribute to the differential stabilities of the three proteins. The COOH-terminal structures, which are thought to control basal turnover, also differ among the three proteins (6, 8, 9). Thus, regulatory mechanisms other than the FWD1-dependent pathway also may contribute to control of the stability of IkB proteins (50). Although the underlying mechanisms responsible for the differential down-regulation of IkB proteins remain to be fully characterized, it is clear that FWD1 is an important regulator of NF-kB signaling.

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REFERENCES

1. May, M. J., and Ghosh, S. (1998) Immunol. Today 19, 80–88
2. Thanos, D., and Maniatis, T. (1995) Cell 80, 529–532
3. Baldwin, A. S., Jr. (1996) Annu. Rev. Immunol. 14, 649–683
4. Ghosh, S., May, M. J., and Kopp, E. B. (1998) Annu. Rev. Immunol. 16, 225–260
5. Kopp, E. B., and Ghosh, S. (1995) Adv. Immunol. 58, 1–27
6. Verma, I. M., Stevenson, J. K., Schwartz, E. M., Van Antwerp, D., and Miyamoto, S. (1995) Genes Dev. 9, 2723–2735
7. Simeonidis, S., Stauber, D., Chen, G., Hendrickson, W. A., and Thanos, D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 49–54
8. Davis, N., Ghosh, S., Simmons, D. L., Tempst, P., Liou, H. C., Baltimore, D., and Nakamura, S. (1995) Mol. Cell. Biol. 15, 2849–2858
9. Haskill, S., Beg, A. A., Liang, S., Chen, G., and Thanos, D. (1997) Genes Dev. 11, 3615–3625
10. Brown, K., Park, S., Kanno, T., Franzoso, G., and Siebenlist, U. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 4982–4986
11. Thompson, J. E., Phillips, R. J., Erdjument-Bromage, H., Tempst, P., and Young, D. B., Barbosa, M., Mann, M., Manning, A., and Rao, A. (1997) Science 278, 860–866
12. Wronczyn, J. D., Gao, X., Cao, Z., Rothe, M., and Goeddel, D. V. (1997) Science 278, 866–869
13. de Martin, R., Vanhove, B., Cheng, Q., Hofer, E., Csizmadia, V., Winkler, H., Harper, J. W. (1995) Genes Dev. 9, 2723–2735
14. de Martin, R., Vanhove, B., Cheng, Q., Hofer, E., Csizmadia, V., Winkler, H., and Bach, F. H. (1995) EMBO J. 14, 2773–2779
15. Le Bail, O., Schmidt-Ullrich, R., and Israel, A. (1995) EMBO J. 14, 5043–5049
16. Chiao, P. J., Miyamoto, S., and Verma, I. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 28–32
17. Thompson, J. E., Phillips, R. J., Erdjument-Bromage, H., Tempst, P., and Ghosh, S. (1995) Cell 80, 573–582
18. Kerr, L. D., Inoue, J., Davis, N., Link, E., Baeuerle, P. A., Bose, H. R., Jr., and Verma, I. M. (1991) Genes Dev. 5, 1464–1476
19. Simeonidis, S., Liang, S., Chen, G., and Thanos, D. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 14372–14377
20. Kunsch, C., and Rosen, C. A. (1993) Mol. Cell. Biol. 13, 6157–6164
21. Malinin, N. L., Boldin, M. P., Kovalenko, A. V., and Wallach, D. (1997) Nature 385, 540–544
22. D’Addamo, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E., and Kirk, H. E., Ray, R. J., and Israel, A. (1998) Cell 91, 243–252
23. Mercier, F., Zhu, H., Murray, N. R., Fields, A. P., and Brasier, A. R. (1999) Mol. Cell. Biol. 19, 243–252
24. Hatakeyama, S., Courtois, G., Bessia, C., Whiteside, S. T. W., Reu, R., Agou, F., Kirk, H. E., Ray, R. J., and Israel, A. (1998) EMBO J. 17, 2431–2440
25. Rothwarf, D. M., Zandi, E., Natoli, G., and Kim, M. (1998) Nature 395, 297–300
26. Cohen, L., Hengel, W. J., and Baeuerle, P. A. (1998) Nature 395, 297–300
27. Chen, Z., Hagler, J., Palombella, V. J., Melanidi, F., Scherer, D., Ballard, D., and Maniatis, T. (1995) Genes Dev. 9, 1566–1597
28. Yaron, A., Gonen, H., Alkalay, I., Hatzubai, A., Jung, S., Beyth, S., Mercurio, F., Manning, A. M., ChecannahVous, and Ben-Neriah, Y. (1997) EMBO J. 16, 6486–6494
29. Hershko, A., and ChecannahVous, and Ben-Neriah, Y. (1992) Annu. Rev. Biochem. 61, 761–807
30. Weissman, A. M. (1997) Immunol. Today 18, 189–198
31. Peters, J. M., Harris, J. R., and Finley, D. (1998) Ubiquitin and Biology of the Cell, Plenum Publishing Corp., New York
32. Elledge, S. J., and Harper, J. W. (1998) Biochem. Biophys. Acta 1377, 61–70
33. Krek, W. (1998) Curr. Opin. Genet. & Dev. 8, 36–42
34. Paton, E. E., Willems, A. R., and Tyers, M. (1998) Trends. Genet. 14, 236–243
35. Hatakeyama, S., Kitagawa, M., Nakayama, K., Shirane, M., Matsumoto, M., Harui, K., Higashi, H., Nakano, H., Okumura, K., Onoe, K., Good, R. A., and Nakayama, K. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3859–3863
36. Kitagawa, M., Hatakeyama, S., Shirane, M., Matsumoto, M., Ishida, N., Hat- tori, K., Nakamichi, K., Kikuchi, A., Nakayama, K.-I., and Nakayama, K. (1997) EMBO J. 16, 2401–2410
37. Yaron, A., Hatzubai, A., Davis, M., Lavon, I., Amit, S., Manning, A. M., Andersen, J. S., Mann, M., Mercurio, F., and Ben-Neriah, Y. (1998) Nature 395, 980–984
38. Winston, J. T., Strack, P., Beer-Romero, P., Chu, C. Y., Elledge, S. J., and Harper, J. W. (1999) Genes Dev. 13, 270–283
39. Spencer, E., Jiang, J., and Chen, Z. J. J. (1999) Genes Dev. 13, 284–294
40. Latres, E., Chiaur, D. S., and Pagano, M. (1999) Oncogene 18, 849–854
41. Fuchs, S. Y., Chen, A., Xiong, Y., Pan, Z. Q., and Ronai, Z. (1999) Oncogene 18, 2039–2046
42. Krek, W., Margottin, F., Kohl, A., Renard, P., Durand, H., Concordet, J.-P., Bacherie, F., Arozaina-Seidesos, F., and Benarous, R. (1999) J. Biol. Chem. 274, 7841–7845
43. Hart, M., Margottin, F., Bourn, S. P., Durand, H., Selig, L., Benichou, S., Richard, V., Thomas, D., Strebel, K., and Benarous, R. (1998) Mol. Cell 1, 565–574
44. Hay, M., Meng, T., Murray, N. R., Fields, A. P., and Brasier, A. R. (1999) J. Biol. Chem. 274, 939–947