A pervasive role of ubiquitin conjugation in activation and termination of I κ B kinase pathways

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The nuclear factor (NF)- κ B pathway is a paradigm for gene expression control by ubiquitin-mediated protein degradation. In stimulated cells, phosphorylation by the I κ B kinase (IKK) complex primes NF- κ B-inhibiting I κ B molecules for lysine (Lys)-48-linked polyubiquitination and subsequent destruction by the 26S proteasome. However, recent studies indicate that the ubiquitin (Ub) system controls NF- κ B pathways at many levels. Ub ligases are activated by different upstream signalling pathways, and they function as central regulators of IKK and c-Jun amino-terminal kinase activation. The assembly of Lys 63 polyUb chains provides docking surfaces for the recruitment of IKK-activating complexes, a reaction that is counteracted by deubiquitinating enzymes. Furthermore, Ub conjugation targets upstream signalling mediators as well as nuclear NF- κ B for post-inductive degradation to limit the duration of signalling.

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Introduction

The vertebrate nuclear factor (NF)- κ B transcription factor family includes NF- κ B1 (p105/p50), NF- κ B2 (p100/p52), p65/RelA, c-Rel and RelB, which function in various biological processes such as apoptosis, proliferation, development and immune response. In resting cells, cytosolic inhibitor- κ B (I κ B) molecules—such as the small proteins I κ B α , β and ϵ or the precursors p100 and p105—inhibit the release of active NF- κ B. Two main signalling pathways have been implicated in receptor-triggered NF- κ B activation. Proinflammatory cytokines, pathogenic stimuli or antigenic peptides induce the canonical NF- κ B pathway with rapid phosphorylation and subsequent degradation of small I κ Bs and p105. The I κ B kinase (IKK) complex, which is composed of the catalytic IKK β and IKK α and the regulatory IKK γ /NF- κ B essential modulator (NEMO) proteins, phosphorylates I κ Bs and p105 at a conserved destruction box. This targets I κ Bs and p105 for lysine

(Lys)-48-linked polyubiquitination (Ub^{Lys48}) by the Skp1–Cullin1–F-box (SCF)/ β -transducin repeat-containing protein (β TrCP) E3-ligase complex and proteasomal destruction, which releases predominantly p50-, p65- and c-Rel-containing heterodimers. With much slower kinetics, a subset of NF- κ B inducers stimulates the IKK α -mediated processing of the precursor p100 and the generation of p52-containing complexes, which also involves ubiquitination and proteasome activity (for reviews, see Bonizzi & Karin, 2004; Hayden & Ghosh, 2004). Although the role of phosphorylation and ubiquitination downstream of IKKs is documented in detail, much less is understood about the post-translational modifications that trigger IKK activation. Recent results suggest a prominent role for the Ub system in activating IKKs in response to tumour necrosis factor- α (TNF- α), interleukin 1 (IL-1), lipopolysaccharide (LPS) and antigen stimulation.

Ub conjugation in the IKK/NF- κ B activation phase

Indications of a proteasome-independent inductive role for ubiquitination upstream of IKKs were first provided by *in vitro* reconstitution experiments (Chen *et al*, 1996). Surprisingly, a Ub-conjugation reaction was required for the activity of a purified IKK complex. TRAF6 (TNF receptor (TNFR)-associated factor 6) contains a RING (really interesting new gene) domain that acts as a Ub ligase (Fig 1). Genetic ablation has demonstrated an essential function of TRAF6 in IKK activation through IL-1 receptor (IL-1R) and Toll-like receptor (TLR) signalling (Lomaga *et al*, 1999). TRAF6 assists the ubiquitin-conjugating E2 enzyme Ubc13/Uev1A in the *in vitro* assembly of Lys-63-linked polyUb chains (Ub^{Lys63}) and the blockade of ubiquitination prevents TRAF6-mediated IKK activation (Deng *et al*, 2000). TRAF6 undergoes trans-auto-ubiquitination (Fig 2A), which is strongly enhanced by its forced oligomerization (Wang *et al*, 2001). TRAF-interacting protein with forkhead-associated domain (TIFA) oligomers promote TRAF6 oligomerization and Ub ligase activity (Ea *et al*, 2004). Furthermore, IKK γ is polyubiquitinated by TRAF6 (Kanayama *et al*, 2004).

TRAF2 and TRAF5 also contain a RING domain (Fig 1) and Ub^{Lys63} modifications can be detected in TRAF2 on TNF- α stimulation (Shi & Kehrl, 2003). In addition, TRAF2 catalyses Lys 63 polyubiquitination of receptor-interacting protein (RIP; Wertz *et al*, 2004). This is an essential pathway component involved in receptor complex assembly in TNF- α -stimulated cells. The severe

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Fig 1 | Components of ubiquitin binding and editing in the IKK/NF- κ B pathways in humans. Ubiquitin (Ub) ligases (E3s) are involved in the activation or termination phases of IKK/NF- κ B signalling. Catalytic domains are coloured green (non-degradative) or red (degradative). Potential Ub-binding domains for Ub^{Lys63} are in light green, and those for degradative polyUb are in purple. Domains with deubiquitinating activity are shown in yellow. BIR, baculoviral inhibition of apoptosis protein repeat; C2, protein kinase C conserved region 2; CAP-Gly, cytoskeleton-associated protein; CARD, caspase recruitment domain; c-IAP1, cellular inhibitor of apoptosis protein; CUE, domain possibly involved in binding to ubiquitin-conjugating enzymes; HECT, homologue of E6-AP carboxyl terminus; Ig, immunoglobulin; MALT1, mucosa-associated lymphoid tissue 1; OTU, ovarian tumour type (cysteine protease); PB1, Phox and Bem1 domain; PLIC, protein linking IAP with cytoskeleton; RING, really interesting new gene; SH2, Src homology 2; SOCS, suppressor of cytokine signalling; STI1, heat-shock chaperonin-binding motif; TAB, TAK1-binding protein; TRABID, TRAF-binding domain; TRAF, TNF-receptor-associated factor domain; β TrCP, transducin-repeat-containing protein; UCH, ubiquitin C-terminal hydrolase; UBA, ubiquitin-associated; UBL, ubiquitin-like; WW, domain with two conserved Trps; ZF, zinc finger; ZF-RBZ, ZF in Ran-binding protein; ZF-ZZ, ZF in dystrophin. Conserved domains were predicted by using <http://smart.embl-heidelberg.de>.

impairment in TNF receptor (TNFR)-induced NF- κ B activation in TRAF2/TRAF5 doubly deficient mice (Tada *et al*, 2001) suggested that TRAF2 and TRAF5 perform a similar function in TNF signalling as TRAF6 has in IL-1 activation. Both TRAF2 and TRAF6 activate c-Jun amino-terminal kinase (JNK) in parallel to IKK, in a process that also depends on the assembly of Ub^{Lys63} chains (Shi & Kehrl, 2003; Wang *et al*, 2001). However, a recent study showed that both TNF- α /TRAF2-mediated IKK and NF- κ B activation require neither the E3 function of the TRAF2 RING domain nor polyUb chains assembled by Ubc13, both of which are needed for JNK activation (Habelhah *et al*, 2004). Notably, both Lys-48- and Lys-63-linked polyUb chains are able to activate JNK, probably by mediating translocation to an insoluble cellular fraction (Habelhah *et al*, 2004). Thus, further analysis and manipulation of

endogenous components is required to solve the obvious discrepancies in the mechanism and function of polyUb chains for IKK versus JNK activation.

Assembly of polyUb chains was also proposed as a crucial step for antigenic signalling to IKK/NF- κ B. T-cell receptor (TCR) ligation triggers protein kinase C θ (PKC θ)-mediated formation of a complex that consists of the CARMA1 (caspase recruitment domain-containing membrane-associated guanylate kinase protein 1), B-cell lymphoma 10 (BCL10) and mucosa-associated lymphoid tissue (MALT1) proteins and transmits the signal to the IKK complex (Fig 2A; Thome, 2004). BCL10 and MALT1 can induce E3 activity of TRAF6, and RNA interference (RNAi) suggests that TRAF2 and TRAF6 are necessary for IKK activation in Jurkat T cells after TCR ligation (Sun *et al*, 2004). However, unequivocal genetic

Fig 2 | The ubiquitin system in the activation and termination phases of IKK/NF- κ B signalling. (A) Ubiquitin (Ub) ligases (shown in green) catalyse the assembly of Ub^{Lys63} chains (green) on TRAF2, TRAF6, RIP and IKK- γ , which is thought to recruit the protein kinase TAK1 through association with TAB2 or TAB3 (light green). TAK1 activates the IKK complex, which subsequently targets I κ Bs for β TrCP-mediated Ub^{Lys48} modification. The ubiquitin-like PLIC may provide a link between the ubiquitination machinery and the proteasome (Kleijne *et al.*, 2000). (B) In the termination phase, DUBs (shown in yellow) catalyse the disassembly of Ub^{Lys63} chains and arrest upstream signalling to IKKs. Distinct E3 Ub ligases (in red) terminate IKK/NF- κ B signalling by the addition of Ub (red) to the substrates for subsequent degradation. Colours for Ub-binding and -editing domains are defined in Fig 1. Abbreviations as in Fig 1; BCL10, B-cell lymphoma 10; CARMA1, caspase recruitment domain-containing membrane-associated guanylate kinase protein 1; PKC, protein kinase C; PLC, phospholipase C.

evidence of a role for TRAF proteins in T-cell activation is missing. Para-caspase MALT1 has Ub ligase activity (Fig 1). MALT1 triggers Ub^{Lys63} modification of Lys 399 in the carboxy-terminal zinc-finger domain of IKK γ ; mutation of this residue abolishes IKK/NF- κ B activation by BCL10 (Zhou *et al.*, 2004). BCL10 induces oligomerization of MALT1 (Lucas *et al.*, 2001), which might enhance its Ub ligase activity. Thus, it can be envisaged that the function of MALT1 after antigenic stimulation resembles the role of TRAFs after pathogen- or cytokine-induced signalling.

Apart from Lys 399, other lysine residues on IKK γ are modified by ubiquitin conjugation in response to signalling. Nucleotide-binding oligomerization domain 2 (NOD2), which functions as an intracellular sensor for bacteria, promotes RIP2-dependent NF- κ B activation by inducing the assembly of Lys 63 polyUb chains at position Lys 285 of IKK γ (Abbott *et al.*, 2004). Genotoxic stress activates NF- κ B by ubiquitination of IKK γ on Lys 277 and Lys 309 (Huang *et al.*, 2003). It is possible that the specific ubiquitin attachment sites on IKK γ may integrate the various upstream signalling pathways.

How is the signal from the Ub^{Lys63}-modified substrates transmitted to the IKK complex and NF- κ B activation? TGF- β -activated kinase 1 (TAK1) is recruited to TRAF6 on IL-1 signalling and activates the IKK/NF- κ B pathway (Ninomiya-Tsuji *et al.*, 1999). TAK1-binding (TAB) proteins are associated with TAK1 and stimulate its kinase activity. Although TAB2-deficient cells have no defect in IL-1 signalling, combined inactivation of TAB2 and TAB3 by RNAi abrogates IKK and JNK/p38 activation by IL-1 and TNF, which suggests functional redundancy (Ishitani *et al.*, 2003). Recently, TAB2

and TAB3 have been shown to act as molecular receptors for Ub^{Lys63}-modified substrates of TRAF2 and TRAF6 (Fig 2A; Kanayama *et al.*, 2004). Both proteins contain C-terminal zinc-finger (ZF-RBZ) motifs (Fig 1), which preferentially associate with Ub^{Lys63} chains and are required for IKK activation. Intriguingly, a replacement of the ZF-RBZ domain with another polyUb-binding motif, such as the p62 ubiquitin-associated (UBA) domain (Fig 1), renders mutant TAB2 capable of mediating IKK activation, which suggests that the recruitment by Ub^{Lys63} chains is required. Thus, as a mechanistic concept, polyubiquitinated TRAF6, IKK γ or RIP recruit TAB2/3-TAK1 complexes and thereby activate TAK1 (Wang *et al.*, 2001). At least *in vitro*, TAK1 can function as an IKK kinase by direct phosphorylation of activation loop serines of IKK β (Wang *et al.*, 2001), and TAK1 downregulation by small interfering RNA (siRNA) interferes with TNF or IL-1 signalling (Takaesu *et al.*, 2003). One mechanism behind this could be the recruitment of putative IKK kinases by Lys 63-modified adaptors through Ub-binding domains. Alternatively, the critical step in IKK activation could be conformational changes that are introduced into the IKK complex by Lys 63 modification and perhaps attraction of Ub-binding proteins.

In addition, Ub^{Lys63} chains regulate the phosphorylation of mitogen-activated protein kinase 6 (MKK6) by TAK1, which activates the JNK and p38 pathways (Wang *et al.*, 2001). However, genetic evidence for the requirement of TAK1 and its adaptors TAB2 and TAB3 in TNFR, IL-1R/TLR and/or TCR signalling is still missing. It is worth noting that TAB2 has also been identified as a component of a nuclear co-repressor complex that is exported

from the nucleus after IL-1 stimulation (Baek *et al*, 2002). How nuclear and cytosolic functions of TAB2 are integrated during signalling remains to be clarified. Interestingly, other polyUb-chain-binding proteins have been shown to affect NF- κ B signalling (Fig 1), and a molecular scaffold function in TNF and IL-1 signalling to IKKs has been proposed for the UBA-domain-containing protein p62 (Sanz *et al*, 2000). Furthermore, p62 gene ablation revealed a requirement for receptor activator of NF- κ B (RANK)-induced osteoclastogenesis and long-term IKK and NF- κ B activation (Duran *et al*, 2004).

Deubiquitination arrests IKK/NF- κ B activation

The concept of a non-degradative function for polyUb in IKK signalling was strengthened by the identification of deubiquitinating enzymes (DUBs) that preferentially disassemble Ub^{lys63} chains (Figs 1,2B). The Ub-specific protease CYLD was identified by its association with IKK γ and through systematic screening for DUBs that would impede NF- κ B signalling (Brummelkamp *et al*, 2003; Kovalenko *et al*, 2003; Trompouki *et al*, 2003). Expression of CYLD is induced by NF- κ B, which provides a novel autoregulatory feedback pathway that could limit the duration of IKK activity (Jono *et al*, 2004). CYLD interferes with IKK/NF- κ B signalling by catalysing the selective cleavage of Ub^{lys63} chains from TRAF2, TRAF6 and IKK γ , without affecting Ub^{lys48}-modified I κ B α or β -catenin (Brummelkamp *et al*, 2003; Kovalenko *et al*, 2003; Trompouki *et al*, 2003). Overexpression of CYLD represses NF- κ B activation in response to various stimuli, including TNF and IL-1. Conversely, CYLD inactivation by RNAi or mutation in the ubiquitin C-terminal hydrolase (UCH) domain increases inducible NF- κ B activity. However, a recent report suggested that CYLD acts in a more pathway-specific manner. In TNF- α -treated cells, CYLD downmodulation selectively enhances JNK but not IKK activation, whereas it equally affects JNK and IKK on stimulation of CD40, LPS or IL-1 (Reiley *et al*, 2004). These data are consistent with findings that TNF- α /TRAF2-mediated polyubiquitination may preferentially activate JNK signalling (Habelhah *et al*, 2004).

Mutations in CYLD cause familial cylindromatosis, which is a rare autosomal-dominant predisposition for developing benign tumours from the proliferative cell types of hair follicles or eccrine glands. Even though the analysis of CYLD in cell lines suggests that it functions as a general negative regulator for IKK and JNK activation by cleaving Ub^{lys63} chains, cylindroma patients display a phenotype that is restricted to epidermal body regions. The TNFR family member ectodysplasin A receptor (EDAR) controls the development of epidermal appendices by activating NF- κ B (Schmidt-Ullrich *et al*, 2001). Therefore, under physiological conditions, the role of CYLD may be restricted to specific cell types and stimulatory conditions, which might point to a functional redundancy with other ubiquitin hydrolases (Fig 1).

With A20, a second IKK-regulating DUB enzyme was recently described. The knockout has revealed a crucial function for limiting inflammation by terminating TNF- α -induced NF- κ B responses (Lee *et al*, 2000). A20 downregulates IKK activation by two mechanisms (Wertz *et al*, 2004). The N-terminal ovarian tumour (OTU) domain of A20 contains DUB activity and catalyses the removal of Ub^{lys63} chains from RIP and TRAF6, thereby interfering with TNFR and TLR signalling to IKKs/NF- κ B, respectively (Boone *et al*, 2004; Wertz *et al*, 2004). Subsequently, the C-terminal zinc-finger domain functions as a Ub ligase that mediates Ub^{lys48}

modification of RIP and initiates its proteasomal degradation (Fig 2; Wertz *et al*, 2004). Using sequence similarity, Cezanne and TRAF-binding domain (TRABID) have been isolated as two further potential DUBs that contain OTU domains (Fig 1) and can interact with TRAF6 (Evans *et al*, 2001). Ectopic expression of Cezanne downregulates NF- κ B, which suggests that Cezanne interferes with IKK activation.

Ub conjugation in the IKK/NF- κ B termination phase

It is well established that the induction of I κ B α expression is required to terminate NF- κ B activation as an autoregulatory feedback mechanism (Hayden & Ghosh, 2004). However, re-synthesis of I κ Bs alone is insufficient to abrogate NF- κ B activity in the presence of continuous upstream signalling. Recent results argue for an important function of the Ub system to limit the duration of NF- κ B signalling by targeted proteolysis in the cytosol and in the nucleus (Fig 2B).

Binding of TNF- α to TNFR1 induces ubiquitination and degradation of RIP after recruitment to lipid rafts (Legler *et al*, 2003) with A20 acting as the potential Ub ligase in this reaction (Wertz *et al*, 2004). In a TNFR2-dependent process that requires the cellular inhibitor of apoptosis protein (c-IAP1) as a Ub ligase, TRAF2 is polyubiquitinated and degraded as well (Li *et al*, 2002). Both gene products, A20 and c-IAP1, are upregulated by NF- κ B, which indicates that an autoregulatory circuit shuts down TNF signalling in a post-inductive manner.

Recent reports have elucidated mechanisms for the downregulation of TCR signalling. Anergic stimuli induce a gene programme in T cells that includes the upregulation of a set of ubiquitin ligases (Heissmeyer *et al*, 2004). Itch, the Ub ligase homologue to the E6-AP C-terminal (HECT) domain, and the closely related Nedd4 protein promote ubiquitination and degradation of phospholipase C γ 1 (PLC γ 1) and PKC θ , both of which are essential mediators of IKK/NF- κ B activation in T cells. Similarly, BCL10, which is downstream of PKC θ on the route to IKKs, is modified by ubiquitination and subsequently degraded after TCR/CD28 co-stimulation of T cells (Scharschmidt *et al*, 2004). Interestingly, the degradation of PLC γ 1, PKC θ and BCL10 is not carried out by the proteasomal machinery and, at least for BCL10, localization to lysosomal vesicles has been observed (Heissmeyer *et al*, 2004; Scharschmidt *et al*, 2004).

The question is whether Ub^{lys63} chains act exclusively in a non-degradative catalytic fashion. p62 has been proposed to act as a shuttling factor to deliver polyubiquitinated substrates to the proteasome. p62 interacts through its UBA domain selectively with Ub^{lys63}-modified proteins, including TRAF6, while the N-terminal Phox and Bem1 (PB1) region provides a docking surface for the proteasome (Seibenhener *et al*, 2004). Thus, under certain conditions, Ub^{lys63} chains might be able to promote proteasomal substrate degradation.

In addition to its contribution to the termination of upstream signalling pathways, the Ub system was proposed to have a role in terminating NF- κ B activity. After TNF- α stimulation, DNA-bound p65 is subject to ubiquitination and proteasomal degradation in the nucleus—a process that seems to be required for abrogation of NF- κ B activity despite I κ B α re-synthesis (Ryo *et al*, 2003; Sacconi *et al*, 2004). It has also been shown that suppressor of cytokine signalling 1 (SOCS1) associates with p65 in LPS-treated cells; the C-terminal SOCS domain functions as an E3 ligase to catalyse the

poly-ubiquitination of p65 and its subsequent degradation (Ryo *et al*, 2003). However, future analyses must define the precise signals and physiological conditions for nuclear degradation of NF- κ B components by SOCS1 or its functional equivalents.

Conclusions and perspectives

The IKK/NF- κ B pathways are classical examples of signalling that involve kinase-induced Ub-dependent protein destruction. Several studies now support a model in which polyubiquitination acts upstream of I κ B kinase activation in NF- κ B signalling. The underlying mechanism is thought to involve ligand-induced oligomerization and activation of Ub ligases, non-destructive Ub^{Lys63} polyubiquitination of adaptors and concomitant recruitment and activation of protein kinases through polyUb-binding domains. The results support a concept in which regulatory ubiquitination is used for signal propagation with a complexity equivalent to, or even exceeding that of, phosphorylation. However, several critical issues still need to be addressed, including: the mapping and mutagenesis of Ub acceptor sites on the various substrate molecules, a detailed comparative analysis of the actions of Lys-63- versus Lys-48-linked polyUb chains, the identification and functional examination of further molecular adaptors for regulatory polyUb chains, and, in particular, the characterization of mechanisms that trigger protein kinase activation on Ub-dependent recruitment. It is possible that polyUb chains provide a surface for the interaction of adaptor molecules, which in turn induce the proximity and/or clustering and (auto)activation of protein kinases (e.g. TAK1, IKK). It will be interesting to see whether such processes could act as a general mechanism for the activation of protein kinases. It can be envisaged that the amplitude and duration of IKK signalling is limited by the activity of deubiquitination enzymes, which disassemble polyUb chains, and by ubiquitin ligases, which trigger post-inductive destruction of upstream adaptors and NF- κ B. After evaluating the specificities of DUBs and E3 ligases in a physiological context, manipulation of these negative regulatory circuits may be a promising strategy for interfering with NF- κ B in cancer or inflammatory diseases.

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REFERENCES

- Abbott DW, Wilkins A, Asara JM, Cantley LC (2004) The Crohn's disease protein, NOD2, requires RIP2 in order to induce ubiquitinylation of a novel site on NEMO. *Curr Biol* **14**: 2217–2227
- Baek SH, Ohgi KA, Rose DW, Koo EH, Glass CK, Rosenfeld M (2002) Exchange of N-CoR corepressor and Tip60 coactivator complexes links gene expression by NF- κ B and β -amyloid precursor protein. *Cell* **110**: 55–67
- Bonizzi G, Karin M (2004) The two NF- κ B activation pathways and their role in innate and adaptive immunity. *Trends Immunol* **25**: 280–288
- Boone DL *et al* (2004) The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. *Nat Immunol* **5**: 1052–1060
- Brummelkamp TR, Nijman SM, Dirac AM, Bernards R (2003) Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF- κ B. *Nature* **424**: 797–801
- Chen ZJ, Parent L, Maniatis T (1996) Site-specific phosphorylation of I κ B α by a novel ubiquitination-dependent protein kinase activity. *Cell* **84**: 853–862
- Deng L, Wang C, Spencer E, Yang L, Braun A, You J, Slaughter C, Pickart C, Chen ZJ (2000) Activation of the I κ B kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* **103**: 351–361
- Duran A, Serrano M, Leitges M, Flores JM, Picard S, Brown JP, Moscat J, Diaz-Meco MT (2004) The atypical PKC-interacting protein p62 is an important mediator of RANK-activated osteoclastogenesis. *Dev Cell* **6**: 303–309
- Ea CK, Sun L, Inoue J, Chen ZJ (2004) TIFA activates I κ B kinase (IKK) by promoting oligomerization and ubiquitination of TRAF6. *Proc Natl Acad Sci USA* **101**: 15318–15323
- Evans PC, Taylor ER, Coadwell J, Heyninck K, Beyaert R, Kilshaw PJ (2001) Isolation and characterization of two novel A20-like proteins. *Biochem J* **357**: 617–623
- Habelhah H, Takahashi S, Cho SG, Kadoya T, Watanabe T, Ronai Z (2004) Ubiquitination and translocation of TRAF2 is required for activation of JNK but not of p38 or NF- κ B. *EMBO J* **23**: 322–332
- Hayden MS, Ghosh S (2004) Signaling to NF- κ B. *Genes Dev* **18**: 2195–2224
- Heissmeyer V, Macian F, Im SH, Varma R, Feske S, Venuprasad K, Gu H, Liu YC, Dustin ML, Rao A (2004) Calcineurin imposes T cell unresponsiveness through targeted proteolysis of signaling proteins. *Nat Immunol* **5**: 255–265
- Huang TT, Wuerzberger-Davis SM, Wu ZH, Miyamoto S (2003) Sequential modification of NEMO/IKK γ by SUMO-1 and ubiquitin mediates NF- κ B activation by genotoxic stress. *Cell* **115**: 565–576
- Ishitani T, Takaes G, Ninomiya-Tsuji J, Shibuya H, Gaynor RB, Matsumoto K (2003) Role of the TAB2-related protein TAB3 in IL-1 and TNF signaling. *EMBO J* **22**: 6277–6288
- Jono H, Lim JH, Chen LF, Xu H, Trompouki E, Pan ZK, Mosialos G, Li JD (2004) NF- κ B is essential for induction of CYLD, the negative regulator of NF- κ B: evidence for a novel inducible autoregulatory feedback pathway. *J Biol Chem* **279**: 36171–36174
- Kanayama A, Seth RB, Sun L, Ea CK, Hong M, Shaito A, Chiu YH, Deng L, Chen ZJ (2004) TAB2 and TAB3 activate the NF- κ B pathway through binding to polyubiquitin chains. *Mol Cell* **15**: 535–548
- Kleijnen MF, Shih AH, Zhou P, Kumar S, Soccio RE, Kederasha NL, Gill G, Howley PM (2000) The hPLIC proteins may provide a link between the ubiquitination machinery and the proteasome. *Mol Cell* **6**: 409–419
- Kovalenko A, Chable-Bessia C, Cantarella G, Israel A, Wallach D, Courtis G (2003) The tumour suppressor CYLD negatively regulates NF- κ B signalling by deubiquitination. *Nature* **424**: 801–805
- Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A (2000) Failure to regulate TNF-induced NF- κ B and cell death responses in A20-deficient mice. *Science* **289**: 2350–2354
- Legler DF, Micheau O, Doucey MA, Tschoep J, Bron C (2003) Recruitment of TNF receptor 1 to lipid rafts is essential for TNF α -mediated NF- κ B activation. *Immunity* **18**: 655–664
- Li X, Yang Y, Ashwell JD (2002) TNF-R1 and c-IAP1 mediate ubiquitination and degradation of TRAF2. *Nature* **416**: 345–347
- Lomaga MA *et al* (1999) TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev* **13**: 1015–1024
- Lucas PC, Yonezumi M, Inohara N, McAllister-Lucas LM, Abazeed ME, Chen FF, Yamaoka S, Seto M, Nunez G (2001) Bcl10 and MALT1, independent targets of chromosomal translocation in malt lymphoma, cooperate in a novel NF- κ B signaling pathway. *J Biol Chem* **276**: 19012–19019
- Ninomiya-Tsuji J, Kishimoto K, Hiyama A, Inoue J, Cao Z, Matsumoto K (1999) The kinase TAK1 can activate the NIK-I κ B as well as the MAP kinase cascade in the IL-1 signaling pathway. *Nature* **398**: 252–256
- Reiley W, Zhang M, Sun S-C (2004) Negative regulation of JNK signaling by the tumor suppressor CYLD. *J Biol Chem* **279**: 55161–55167
- Ryo A, Suizu F, Yoshida Y, Perrem K, Liou YC, Wulf G, Rottapel R, Yamaoka S, Lu KP (2003) Regulation of NF- κ B signaling by Pin1-dependent prolyl isomerization and ubiquitin-mediated proteolysis of p65/RelA. *Mol Cell* **12**: 1413–1426
- Saccani S, Marazzi I, Beg AA, Natoli G (2004) Degradation of promoter-bound p65/RelA is essential for the prompt termination of the nuclear factor κ B response. *J Exp Med* **200**: 107–113
- Sanz L, Diaz-Meco MT, Nakano H, Moscat J (2000) The atypical PKC-interacting protein p62 channels NF- κ B activation by the IL-1–TRAF6 pathway. *EMBO J* **19**: 1576–1586
- Scharschmidt E, Wegener E, Heissmeyer V, Rao A, Krappmann D (2004) Degradation of Bcl10 induced by T-cell activation negatively regulates NF- κ B signaling. *Mol Cell Biol* **24**: 3860–3873

- Schmidt-Ullrich R, Aebischer T, Hulsken J, Birchmeier W, Klemm U, Scheidereit C (2001) Requirement of NF- κ B/Rel for the development of hair follicles and other epidermal appendages. *Development* **128**: 3843–3853
- Seibenhener ML, Babu JR, Geetha T, Wong HC, Krishna NR, Wooten MW (2004) Sequestosome 1/p62 is a polyubiquitin chain binding protein involved in ubiquitin proteasome degradation. *Mol Cell Biol* **24**: 8055–8068
- Shi CS, Kehrl JH (2003) Tumor necrosis factor (TNF)-induced germinal center kinase-related (GCKR) and stress-activated protein kinase (SAPK) activation depends upon the E2/E3 complex Ubc13–Uev1A/TNF receptor-associated factor 2 (TRAF2). *J Biol Chem* **278**: 15429–15434
- Sun L, Deng L, Ea CK, Xia ZP, Chen ZJ (2004) The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol Cell* **14**: 289–301
- Tada K *et al* (2001) Critical roles of TRAF2 and TRAF5 in tumor necrosis factor-induced NF- κ B activation and protection from cell death. *J Biol Chem* **276**: 36530–36534
- Takaesu G, Surabhi RM, Park KJ, Ninomiya-Tsuji J, Matsumoto K, Gaynor RB (2003) TAK1 is critical for I κ B kinase-mediated activation of the NF- κ B pathway. *J Mol Biol* **326**: 105–115
- Thome M (2004) CARMA1, BCL-10 and MALT1 in lymphocyte development and activation. *Nat Rev Immunol* **4**: 348–359
- Trompouki E, Hatzivassiliou E, Tschritzis T, Farmer H, Ashworth A, Mosialos G (2003) CYLD is a deubiquitinating enzyme that negatively regulates NF- κ B activation by TNFR family members. *Nature* **424**: 793–796
- Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ (2001) TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* **412**: 346–351
- Wertz IE *et al* (2004) De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF- κ B signalling. *Nature* **430**: 694–699
- Zhou H, Wertz I, O'Rourke K, Ultsch M, Seshagiri S, Eby M, Xiao W, Dixit VM (2004) Bcl10 activates the NF- κ B pathway through ubiquitination of NEMO. *Nature* **427**: 167–171

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