Inducible SUMO modification of TANK alleviates its repression of TLR7 signalling

Florian Renner^{1*}, Vera V. Saul^{1*}, Axel Pagenstecher², Tobias Wittwer¹ & Michael Lienhard Schmitz¹⁺

¹Institute of Biochemistry, Medical Faculty, Justus-Liebig-University, Giessen, and ²Department of Neuropathology, University of Marburg, Marburg, Germany

Adaptor proteins allow temporal and spatial coordination of signalling. In this study, we show SUMOylation of the adaptor protein TANK and its interacting kinase TANK-binding kinase 1 (TBK1). Modification of TANK by the small ubiquitin-related modifier (SUMO) at the evolutionarily conserved Lys282 is triggered by the kinase activities of I κ B kinase ϵ (IKK ϵ) and TBK1. Stimulation of TLR7 leads to inducible SUMOylation of TANK, which in turn weakens the interaction with IKK ϵ and thus relieves the negative function of TANK on signal propagation. Reconstitution experiments show that an absence of TANK SUMOylation impairs inducible expression of distinct TLR7-dependent target genes, providing a molecular mechanism that allows the control of TANK function.

Keywords: SUMO; TANK; TLR signalling; NF- κ B; transcription
EMBO reports (2011) 12, 129–135. doi:10.1038/embor.2010.207

INTRODUCTION

The NF- κ B transcription factor system has evolved to mediate a ubiquitous stress response by inducing the transcription of antiapoptotic and proinflammatory genes. NF- κ B activity must be tightly controlled, as compromised NF- κ B function results in immunodeficiency and excessive activation leads to an exaggerated inflammatory response that might become chronic (Oeckinghaus & Ghosh, 2009; Renner & Schmitz, 2009). The main step in canonical NF- κ B activation is the phosphorylation and ubiquitin/proteasome-dependent elimination of inhibitory I κ B proteins, which retain the DNA-binding subunits in the cytosol of unstimulated cells. I κ B phosphorylation is mediated by the canonical IKK complex that consists of IKK α , IKK β and the adaptor protein NF- κ B essential modulator (NEMO) (IKK γ ; Vallabhapurapu & Karin, 2009).

The NF- κ B activation pathway uses several adaptor proteins, which regulate the kinetics and thresholds of signalling pathways

(Kholodenko *et al*, 2010). Adaptor and scaffolding proteins typically lack intrinsic enzymatic activities, but bind to at least two other signalling proteins and thus act as platforms that allow the coordinated and ordered assembly of signalling complexes (Shaw & Filbert, 2009). In this manner, adaptor proteins help to define the quality of the signal output and coordinate signal specificity. The adaptor protein TANK—also known as TRAF-interacting protein (I-TRAF)—has been identified as a TRAF-binding protein (Cheng & Baltimore, 1996; Rothe *et al*, 1996). The analysis of TANK-deficient mice showed a pathway-specific function of this adaptor protein, as its elimination selectively increased TLR7-triggered NF- κ B and AP1 activity (Kawagoe *et al*, 2009). TANK binds to the IKK-related non-canonical I κ B kinases TBK1 and IKK ϵ , which control IRF3/7 target gene transcription and also contribute to the regulation of NF- κ B transactivation (Chau *et al*, 2008). A yeast two-hybrid screen identified the direct binding of TANK to NEMO (Chariot *et al*, 2002), an adaptor protein that, in contrast to TANK, does not function as a pathway-specific negative regulator, but functions as a platform for activation of the IKK complex (Israel, 2010). Although TANK functions to restrict TLR7-induced ubiquitination of TRAF6, regulation of the inhibitory activity of TANK is unknown. The two TANK-binding proteins NEMO and IKK ϵ can be post-translationally modified by covalent coupling to the small ubiquitin-related modifier (SUMO). Similarly to ubiquitin, SUMO is conjugated to a substrate protein lysine by an isopeptide bond in an ATP-consuming enzymatic reaction that uses E1, E2 and E3 enzymes (Wilkinson & Henley, 2010). Protein SUMOylation is a dynamic process, as the activity of SUMO E3 ligases is antagonized by SUMO isopeptidases (Hay, 2007). In contrast to degradative ubiquitination, SUMOylation of proteins does not target them for proteolysis, but rather controls protein–protein interactions, conformation and intracellular localization of the modified substrates (Geiss-Friedlander & Melchior, 2007).

RESULTS AND DISCUSSION

The identification of IKK ϵ as a SUMO substrate (Renner *et al*, 2010) prompted us to test whether this post-translational modification also occurs for TBK1 and TANK. Coexpression of His-tagged SUMO1 with TANK and either IKK ϵ or TBK1 allowed us to detect SUMOylated TANK in eluates from nickel-nitrilotriacetic

¹Institute of Biochemistry, Medical Faculty, Justus-Liebig-University, Friedrichstrasse 24, Giessen 35392, Germany

²Department of Neuropathology, University of Marburg, Baldingerstrasse, Marburg D-35043, Germany

*These authors contributed equally to this work

+Corresponding author. Tel: +49 641 994 7570; Fax: +49 641 994 7589; E-mail: lienhard.schmitz@biochemie.med.uni-giessen.de

Fig 1 | Kinase-dependent SUMOylation of TANK. (A) HEK293T cells were transfected to express TANK along with His-tagged SUMO1 and Flag-tagged versions of active or inactive IKKε and TBK1 as shown. A fraction of cells was lysed in 1 × SDS sample buffer and used for the analysis of protein expression and the phosphorylation of TANK and the positive control IRF3 with specific antibodies by immunoblotting. Another fraction of cells was lysed in denaturing Gu-HCl lysis buffer to maintain SUMOylation, followed by the enrichment of SUMOylated proteins on Ni-NTA agarose beads. Eluates were analysed for the occurrence and SUMOylation of TANK, the position of a non-specific band is indicated. (B) Tagged versions of IKKε and TBK1 were expressed together with His-SUMO1 or a point mutant that cannot be attached to its substrate (His-SUMO1-G97A). Expression and SUMO modification of proteins were analysed as in (A) either by direct lysis in 1 × SDS sample buffer or after enrichment of SUMO-modified proteins on Ni-NTA columns. (C) IKKε and His-SUMO1 were expressed either with TANK or a phosphorylation-deficient TANK mutant. Phosphorylation and SUMOylation of the TANK proteins were revealed with specific antibodies as shown. (D) Cells were transfected with the indicated expression vectors together with wild-type and point-mutated TANK. Immunoblotting showed SUMOylation of TANK in 1 × SDS lysates and also after enrichment of SUMO-modified proteins on Ni-NTA columns. HA, haemagglutinin; IB, immunoblotting; IKKε, IκB kinase ε; Ni-NTA, nickel nitrilotriacetic acid; NS, non-specific; SUMO, small ubiquitin-related modifier.

acid (Ni-NTA) columns (Fig 1A). SUMO modification of TANK was strongly augmented by active IKKε or TBK1, whereas kinase-inactive versions failed to enhance SUMOylation of TANK. Similarly, expression of TBK1 with His-tagged SUMO1 allowed the enrichment of SUMOylated TBK1 on Ni-NTA columns (Fig 1B), but, in contrast to TANK, this SUMOylation was not increased in the presence of IKKε. SUMO modification of TBK1 and IKKε was dependent on the integrity of the carboxy-terminal glycine of SUMO1 that is conjugated to the lysine in the target protein. In summary, these data show that both non-canonical IKKs and their adaptor protein TANK can be modified by SUMOylation, indicating that other scaffolding components for the non-canonical IKKs, such as NAK-associated protein (NAP1) or Sintbad, might be regulated by the SUMO system. To investigate the role of IKKε kinase activity in the SUMOylation of TANK, the wild-type form of IKKε was compared with

enzymatically inactive point mutants that are changed either in the ATP-binding pocket (IKKε-K38A) or in the activation loop (IKKε-S172A). Analysis of TANK SUMOylation after coexpression of the respective IKKε variants showed that IKKε kinase activity is necessary for TANK modification (Fig 1A; supplementary Fig S1 online). TANK can be phosphorylated by IKKε (Nomura *et al*, 2000) and also by TBK1 (Gatot *et al*, 2007), raising the question whether the kinase dependence of TANK SUMOylation is due to direct phosphorylation of TANK or to phosphorylation of other targets. To distinguish between these possibilities, a non-phosphorylatable TANK mutant was created. For this mutant, all known phosphorylation sites (<http://www.phospho.org>) and putative IKKε phosphorylation motifs (Hutti *et al*, 2009) were changed to alanines. The comparison between TANK and the mutated TANK form (TANK31xST/A) showed intact IKKε-dependent SUMOylation of TANK31xST/A, although kinase-dependent

phosphorylation was mostly lost (Fig 1C). Similarly, TBK1-dependent TANK SUMOylation still occurred for the phosphorylation-deficient mutant (supplementary Fig S2 online). These data indicate that the kinase dependence of TANK SUMOylation is not due to direct phosphorylation of the substrate protein, but rather due to phosphorylation of other targets such as components of the enzymatic machinery regulating the attachment or deconjugation of SUMO. To map the SUMO acceptor lysine of TANK, candidate lysines contained in SUMO consensus motifs were changed to arginines. SUMOylation of TANK was reduced on mutagenesis of Lys282 (Fig 1D), thus identifying this evolutionarily conserved residue (supplementary Fig S3 online) as the SUMO attachment site. The SUMOylated lysine is not flanked by a proline-directed phosphorylation site, as it occurs frequently in proteins in which these two post-translational modifications are coupled (Hietakangas *et al*, 2006).

To test the ability of IKK ϵ SUMOylation to promote TANK SUMOylation, cells were transfected to express TANK and His-tagged SUMO1 along with IKK ϵ , the non-SUMOylatable IKK ϵ mutant IKK ϵ -K231R or a SUMO fusion thereof. Although IKK ϵ strongly stimulated SUMO modification of TANK, the kinase-active but SUMOylation-defective IKK ϵ -K231R mutant failed to trigger TANK modification. This defect was partly rescued on genetic fusion of IKK ϵ -K282R and SUMO1 (Fig 2A), showing that not only kinase activity but also SUMOylation of IKK ϵ is required for its stimulatory function on TANK modification. SUMOylation of TANK was also enhanced by the SUMO E2 enzyme Ubc9, whereas coexpression of the isopeptidase sentrin-specific protease-1 (SEN1) resulted in SUMO deconjugation (Fig 2B). Further experiments showed that protein inhibitor of activated STATy (PIASy) selectively and dose-dependently augmented TANK SUMOylation, while other E3 ligases such as Pc2 had no effect (Fig 2C). By contrast, a catalytically inactive PIASy mutant (PIASy-C/A) failed to stimulate TANK SUMOylation and even suppressed basal SUMO modification (Fig 2D). Knockdown of endogenous PIASy by short interfering RNA (siRNA) resulted in an impaired SUMOylation of TANK (Fig 2E), suggesting an important contribution of PIASy to the SUMO modification of TANK.

The two interacting adaptor proteins TANK and NEMO are SUMOylated and both use PIASy as a common SUMO E3 ligase (Mabb *et al*, 2006). These shared features prompted us to test whether IKK ϵ also has the ability to trigger SUMOylation of NEMO. IKK ϵ enhanced SUMO modification of NEMO in a kinase-dependent manner (Fig 3A), an effect that was lost on coexpression of TANK (Fig 3B). Conversely, expression of NEMO impaired SUMOylation of TANK (Fig 3C), showing that there is competition between adaptor proteins for SUMO modification. Many adaptor proteins, such as NEMO, are not only covalently bound to ubiquitin or ubiquitin-like proteins, but also have the ability to interact with these moieties in a non-covalent manner to create protein-protein interaction surfaces. To test whether TANK also has the ability to bind to ubiquitin or SUMO, glutathione-S-transferase (GST) pull-down experiments were performed (Fig 3D). Bacterially expressed and purified GST-SUMO1, GST-ubiquitin and GST-4x ubiquitin failed to interact with TANK, while NEMO was efficiently captured by linear ubiquitin chains represented by GST-4x ubiquitin, in accordance with previous data (Rahighi *et al*, 2009). Binding of TANK to linear ubiquitin chains only occurred on coexpression of NEMO, showing that TANK lacks an obvious

SUMO or ubiquitin-binding domain but can indirectly associate with linear ubiquitin chains on interaction with NEMO. Given the relevance of TANK for TLR7 signalling, we tested for a TLR7-dependent regulation of TANK SUMOylation in HEK293 cells stably expressing TLR7. These cells were transiently transfected to express moderate amounts of TANK and IKK ϵ together with SUMO2, as this paralogue can be conjugated in a more dynamic manner than SUMO1 (Golebiowski *et al*, 2009) and is also attached to TANK (supplementary Fig S4 online). Stimulation of cells with the synthetic TLR7 ligand R-848 allowed the detection of a strongly induced TANK SUMOylation, which reached its maximum 2 h after stimulation and then dropped to normal levels (Fig 4A). To investigate regulated TANK SUMOylation in a physiological setting, Ramos cells were used to study dynamic SUMOylation of endogenous TANK. As permanent isopeptidase-mediated cleavage of SUMO chains impedes the detection of this post-translational modification in standard lysis buffers (Wilkinson & Henley, 2010), cells expressing His-tagged SUMO2 were treated for specific times with R-848 and then lysed under denaturing conditions. After enrichment of SUMOylated proteins on Ni-NTA agarose, immunoblotting showed increased SUMOylation of endogenous TANK 2 h after TLR7 stimulation (Fig 4B). To test the effect of SUMOylation on protein-protein interactions occurring *in vivo*, cells expressing either TANK or the non-SUMOylatable TANK-K282R mutant were treated with a membrane-permeable crosslinker to allow covalent crosslinking of proteins that are in close proximity. Coimmunoprecipitation experiments detected an augmented interaction between IKK ϵ and TANK-K282R (Fig 4C), indicating an inhibitory function of TANK modification on its ability to bind IKK ϵ . On the basis of the identification of several TANK-controlled genes such as *Cox-2* (Kawagoe *et al*, 2009), it was interesting to study the effect of TANK SUMOylation on the expression of such gene products. TLR7-expressing HEK293 cells were transfected with a vector for short-hairpin RNA (shRNA)-mediated knockdown of endogenous TANK together with codon-exchanged, and thus shRNA-resistant, versions of TANK or TANK-K282R (supplementary Fig S5 online). R-848-triggered expression of *Cox2* was exaggerated in the absence of TANK and impaired on prevention of TANK SUMOylation (Fig 4D), revealing an activating role of TANK SUMOylation as shown for the expression of *Cox2*. The negative effect of TANK on TLR7 signalling creates a need to relieve this inhibitory activity after receptor stimulation. Our data suggest that IKK ϵ /TBK1-mediated phosphorylation events enable inducible SUMOylation of TANK, which in turn disturbs the interaction with IKK ϵ to allow TRAF-dependent signal propagation, as schematically shown in Fig 4E. A previous study showed that IKK ϵ -mediated phosphorylation of TRAF2 inhibits mutual binding between both proteins (Nomura *et al*, 2000), suggesting that activating signals lead to the disruption of protein complexes containing the inhibitory TANK protein. In this study, we describe regulation of TANK SUMOylation by TLR7 activation, and other signals that might affect TANK modification. The reported upregulation of SUMO2 expression in heat-shock conditions (Golebiowski *et al*, 2009) prompted us to test the consequences of temperature increases on the modification of TANK by SUMO2. These experiments showed elevated SUMOylation of TANK on temperature increase (supplementary Fig S6 online), which might be a mechanism to regulate TANK activity during fever. TANK is a classic scaffold protein that binds

Fig 2 | SENP1 and PIASy regulate TANK SUMOylation. (A) Cells were transfected to express TANK and His-SUMO1 with wild-type or SUMOylation-defective IKK ϵ as shown. Expression and modification of proteins was analysed after lysis in 1 × SDS sample buffer or after enrichment of SUMOylated proteins on Ni-NTA columns. (B) Cells were transfected with vectors encoding TANK and IKK ϵ together with epitope-tagged versions of SUMO1, Ubc9 and SENP1 as shown. His-SUMO1-modified proteins were enriched on Ni-NTA columns and the level of TANK SUMOylation was shown by western blotting. Immunoblot analysis of 1 × SDS lysates confirmed adequate protein expression. (C) HEK293T cells were transfected to express epitope-tagged TANK, IKK ϵ , SUMO1 and different amounts of the SUMO E3 ligases PIASy or Pc2 as indicated. Cell lysates were analysed for the relative SUMOylation levels of TANK by immunoblotting. (D) Changes in the SUMOylation status of Flag-TANK induced by the coexpression of HA-PIASy or catalytically inactive HA-PIASy-C/A were analysed by western blotting. (E) Cells transfected with a PIASy-specific siRNA or a control were used for the analysis of TANK SUMOylation (left) after coexpression of TANK with His-SUMO1 and Flag-IKK ϵ as shown. In the absence of antibodies recognizing the endogenous PIASy protein, an aliquot of the cells was used to test mRNA expression of PIASy by qPCR (right) or for downregulation of epitope-tagged PIASy (supplementary Fig S7 online). Bars show values of s.d. GFP, green fluorescent protein; HA, haemagglutinin; IKK ϵ , I κ B kinase ϵ ; mRNA, messenger RNA; Ni-NTA, nickel nitrilotriacetic acid; NS, non-specific; PIASy, protein inhibitor of activated STATy; qPCR, quantitative PCR; siRNA, short interfering RNA; SUMO, small ubiquitin-related modifier.

Fig 3 | SUMO modification of NEMO is modulated by TANK and IKK ϵ . (A) HEK293T cells were transfected to express Flag-NEMO along with the indicated versions of IKK ϵ and SUMO1. A fraction of cells was directly lysed in 1 × SDS sample buffer and used for input controls. SUMOylation of NEMO was examined after enrichment on Ni-NTA matrices. (B) Transfected HEK293T cells were lysed in either Gu-HCl or 1 × SDS sample buffer. SUMOylated proteins contained in Gu-HCl lysates were enriched on Ni-NTA beads and eluted. SUMO modification and expression of proteins was analysed with specific antibodies as shown. (C) Cells were transfected to trigger SUMOylation of TANK in the absence or presence of coexpressed NEMO as shown. Expression of proteins and SUMO modification of TANK were shown by western blotting. (D) Cells were transfected to express the indicated combination of expression vectors and lysed in NP-40 buffer. Cell extracts were incubated with the indicated GST fusion proteins and GSH sepharose. GST pull-down experiments were performed and the input material and the eluates were analysed by immunoblotting. The lower part shows the Coomassie-stained GST fusion proteins used for this experiment. GSH, glutathione; GST, glutathione-S-transferase; IKK ϵ , I κ B kinase ϵ ; NEMO, NF- κ B essential modulator; Ni-NTA, nickel-nitrilotriacetic acid; SUMO, small ubiquitin-related modifier.

to other signalling proteins and acts as a platform to coordinate and specify signalling pathways (Shaw & Filbert, 2009), which raises the need to control its function. This can be achieved by SUMOylation—as shown in this study—and also by other modifications such as phosphorylation and ubiquitination, both of which require TBK1 or IKK ϵ (Nomura *et al*, 2000; Bonif *et al*, 2006; Gatot *et al*, 2007).

METHODS

The TANK-31xST/A construct was produced on mutation of serines and threonines at positions 49, 67, 68, 116, 117, 126, 129, 164, 175, 178, 208, 213, 224, 225, 228, 230, 243, 244, 248, 249, 257, 260, 261, 286, 298, 341, 354, 357, 380, 406 and 409 by constructing a synthetic gene (Geneart) and recloning into a pCMV-Flag expression vector. The TANK knockdown plasmid pSUPER-puro-shTANK with the targeting sequence 5'-AGACT GAGAACTATGAGCA-3' was cloned by standard procedures. The siRNA used for the knockdown of PIASy was purchased from Qiagen (Hs_PIAS4_3 CACCGAATTAGTCCCACAGAA), of which 5 nM of it was transfected into the cells using the HiPerfect

reagent. Details on further plasmids are available as supplementary information online.

Crosslinking and immunoprecipitation. Cells were incubated for 30 min in PBS buffer supplemented with the cell-permeable crosslinker dimethyl-3-3'-dithiobispropionimidate 2-HCl (0.5 mM). After quenching by an incubation in PBS/200 mM Tris-HCl (pH 7.5), cells were lysed for 20 min on ice using NP-40 buffer and used for immunoprecipitation, essentially as described (Rosic *et al*, 2006).

Cell extracts and western blotting. Depending on the experimental setting, cells were lysed in different buffers. To analyse substrate SUMOylation, cells were directly lysed in 1 × SDS buffer, followed by shearing of DNA using a Branson sonifier, essentially as described. Alternatively, cells were lysed in Gu-HCl lysis buffer to allow purification on Ni-NTA columns or in NP-40 buffer as described (Renner *et al*, 2010).

Statistical analysis. All figures are representative of at least three independent experiments. Real-time PCR data are shown as the means of four independent experiments measured in triplicate and error bars represent the s.e.m.

Fig 4 | SUMOylation relieves TANK-mediated repression of TLR7 signalling. (A) Cells stably expressing TLR7 were transfected to express His-SUMO2, GFP-IKKε and TANK as shown. After 36 h, cells were stimulated with R-848 for the indicated times and lysed. SUMOylation of TANK was analysed after purification of SUMOylated proteins on Ni-NTA columns, and correct protein expression was ensured in material that was lysed in 1 × SDS buffer. (B) Ramos cells were electroporated to express His-SUMO2 as shown. After 2 days, cells were stimulated with R-848 for the indicated times. A fraction of cells was lysed in NP-40 buffer and used for the input controls, while SUMOylation of endogenous TANK was analysed after purification of SUMOylated proteins on Ni-NTA beads. (C) Cells transiently expressing IKKε along with wild-type or SUMOylation-defective TANK were treated for 30 min with a cell-permeable crosslinker to preserve protein-protein interactions occurring in intact cells. After removal of the crosslinker and quenching, cells were lysed and immunoprecipitated using a Flag antibody. The precipitates and input controls were analysed by immunoblotting. (D) Cells stably expressing TLR7 were transfected with a vector encoding a short-hairpin RNA specific for endogenous TANK together with codon-exchanged and therefore short-hairpin RNA-resistant versions of TANK or TANK-K282R and selected for 3 days with puromycin as shown. Cells were stimulated for 8 h with R-848, followed by detection of TANK, Cox2 and tubulin with specific antibodies as shown. (E) Model for the role of inducible TANK SUMOylation. GFP, green fluorescent protein; IB, immunoblotting; IKKε, IκB kinase ε; IP, immunoprecipitation; mut, mutant; Ni-NTA, nickel nitrilotriacetic acid; SUMO, small ubiquitin-related modifier.

Further methods are available as supplementary information online.
Supplementary information is available at EMBO reports online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

We thank Drs Alain Chariot (Université de Liège, Belgium), Ivan Dikic (University of Frankfurt, Germany), Ron T. Hay (University of Dundee, UK), Daniel Krappmann (Forschungszentrum für Umwelt und Gesundheit, Munich, Germany), Shigeki Miyamoto (University of Wisconsin, USA) and Hans Will (Heinrich-Pette-Institute Hamburg, Germany) for generously providing the plasmids. We are also grateful to Dr Stefan Bauer (Marburg, Germany) for supplying HEK293 cells stably expressing TLR7 and to Daniela Stock for excellent technical assistance. Work in our lab is supported by grants from the Deutscher Akademischer Austausch Dienst, Humboldt Foundation, Deutsche Forschungsgemeinschaft, the Excellence Cluster Cardio-Pulmonary System and the Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz program.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

- Bonif M *et al* (2006) TNFα- and IKKβ-mediated TANK/I-TRAF phosphorylation: implications for interaction with NEMO/IKKγ and NF-κB activation. *Biochem J* **394**: 593–603
- Chariot A, Leonardi A, Muller J, Bonif M, Brown K, Siebenlist U (2002) Association of the adaptor TANK with the IκB kinase (IKK) regulator NEMO connects IKK complexes with IKKε and TBK1 kinases. *J Biol Chem* **277**: 37029–37036
- Chau TL, Gioia R, Gatot JS, Patrascu F, Carpentier I, Chapelle JP, O'Neill L, Beyaert R, Piette J, Chariot A (2008) Are the IKKs and IKK-related kinases TBK1 and IKK-ε similarly activated? *Trends Biochem Sci* **33**: 171–180
- Cheng G, Baltimore D (1996) TANK, a co-inducer with TRAF2 of TNF- and CD 40L-mediated NF-κB activation. *Genes Dev* **10**: 963–973
- Gatot JS *et al* (2007) Lipopolysaccharide-mediated interferon regulatory factor activation involves TBK1-IKKε-dependent Lys(63)-linked polyubiquitination and phosphorylation of TANK/I-TRAF. *J Biol Chem* **282**: 31131–31146
- Geiss-Friedlander R, Melchior F (2007) Concepts in sumoylation: a decade on. *Nat Rev Mol Cell Biol* **8**: 947–956
- Golebiowski F, Matic I, Tatham MH, Cole C, Yin Y, Nakamura A, Cox J, Barton GJ, Mann M, Hay RT (2009) System-wide changes to SUMO modifications in response to heat shock. *Sci Signal* **2**: ra24
- Hay RT (2007) SUMO-specific proteases: a twist in the tail. *Trends Cell Biol* **17**: 370–376

- Hietakangas V, Anckar J, Blomster HA, Fujimoto M, Palvimo JJ, Nakai A, Sistonen L (2006) PDSM, a motif for phosphorylation-dependent SUMO modification. *Proc Natl Acad Sci USA* **103**: 45–50
- Hutti JE, Shen RR, Abbott DW, Zhou AY, Spratt KM, Asara JM, Hahn WC, Cantley LC (2009) Phosphorylation of the tumor suppressor CYLD by the breast cancer oncogene IKK ϵ promotes cell transformation. *Mol Cell* **34**: 461–472
- Israel A (2010) The IKK complex, a central regulator of NF- κ B activation. *Cold Spring Harb Perspect Biol* **2**: a000158
- Kawagoe T, Takeuchi O, Takabatake Y, Kato H, Isaka Y, Tsujimura T, Akira S (2009) TANK is a negative regulator of Toll-like receptor signaling and is critical for the prevention of autoimmune nephritis. *Nat Immunol* **10**: 965–972
- Kholodenko BN, Hancock JF, Kolch W (2010) Signalling ballet in space and time. *Nat Rev Mol Cell Biol* **11**: 414–426
- Mabb AM, Wuerzberger-Davis SM, Miyamoto S (2006) PIASy mediates NEMO sumoylation and NF- κ B activation in response to genotoxic stress. *Nat Cell Biol* **8**: 986–993
- Nomura F, Kawai T, Nakanishi K, Akira S (2000) NF- κ B activation through IKK-i-dependent I-TRAF/TANK phosphorylation. *Genes Cells* **5**: 191–202
- Oeckinghaus A, Ghosh S (2009) The NF- κ B family of transcription factors and its regulation. *Cold Spring Harb Perspect Biol* **1**: a000034
- Rahighi S *et al* (2009) Specific recognition of linear ubiquitin chains by NEMO is important for NF- κ B activation. *Cell* **136**: 1098–1109
- Renner F, Schmitz ML (2009) Autoregulatory feedback loops terminating the NF- κ B response. *Trends Biochem Sci* **34**: 128–135
- Renner F, Moreno R, Schmitz ML (2010) SUMOylation-dependent localization of IKK ϵ in PML nuclear bodies is essential for protection against DNA-damage-triggered cell death. *Mol Cell* **37**: 503–515
- Roscic A, Moller A, Calzado MA, Renner F, Wimmer VC, Gresko E, Ludi KS, Schmitz ML (2006) Phosphorylation-dependent control of Pc2 SUMO E3 ligase activity by its substrate protein HIPK2. *Mol Cell* **24**: 77–89
- Rothe M, Xiong J, Shu HB, Williamson K, Goddard A, Goeddel DV (1996) I-TRAF is a novel TRAF-interacting protein that regulates TRAF-mediated signal transduction. *Proc Natl Acad Sci USA* **93**: 8241–8246
- Shaw AS, Filbert EL (2009) Scaffold proteins and immune-cell signalling. *Nat Rev Immunol* **9**: 47–56
- Vallabhapurapu S, Karin M (2009) Regulation and function of NF- κ B transcription factors in the immune system. *Annu Rev Immunol* **27**: 693–733
- Wilkinson KA, Henley JM (2010) Mechanisms, regulation and consequences of protein SUMOylation. *Biochem J* **428**: 133–145