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Review

SUMO and NF-κB ties

A. M. Mabb and S. Miyamoto*

Program in Molecular and Cellular Pharmacology, Department of Pharmacology, University of Wisconsin, 301 Medical Sciences Center, 1300 University Avenue, Madison, WI 53706 (USA), Fax: +1 608 262 1257, e-mail: smiyamot@wisc.edu

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Abstract. Members of the NF-κB family of transcription factors play critical roles in regulating immunity and cell survival and contribute to cancer progression and chemoresistance. Over the past 20 years, much has been learned about the remarkable complexity in regulation of NF-κB signaling. In particular, recent studies have added to our current understanding of the roles of a multitude of post-translational modifications in this signaling system: these include phosphorylation, acetylation, nitrosylation, ubiquitination, neddylation and sumoylation. This review will highlight our current knowledge of the roles of sumoylation in regulating NF-κB signaling and functions and will address future perspectives.

Keywords. SUMO, NF-kappaB, posttranslational, transcription, Ubc9, PIAS, NEMO, IkappaBalpha.

Overview of the NF-κB signaling system

It has been more than 20 years since the initial report of the discovery of the transcription factor nuclear factor kappa B (NF-κB) [1]. NF-κB refers to a collection of protein dimers, each composed of members of the NF-κB/Rel family of proteins. In the mammalian system, there are five members: p65/RelA, RelB, c-Rel, p105/p50 and p100/p52. Conserved members of this family can also be found in other eukaryotic systems, including Drosophila (Dorsal, Dif and Relish), cnidarians (Nv-NF-κB) and others [2]. In mammalian cells, the most ubiquitous NF-κB dimer consists of a p65/p50 heterodimer [3] that is found in the cytoplasm bound to members of a family of inhibitors known as inhibitor of NF-κB (IκB). The binding of IκB family members, such as IκBα and IκBβ, can prevent nuclear accumulation of NF-κB, in part *via* masking the nuclear localization sequence (NLS) of NF-κB [4, 5]. IκBα can also promote nuclear export of bound NF-κB, further ensuring cytoplasmic localization of inactive NF-κB dimers [6–10].

Activation of NF-κB involves its release from IκB, followed by nuclear translocation and a multitude of posttranslational modifications that enable its transcriptional activation function. In this review, the term "signaling" (or "signal transduction") is used specifically to refer to molecular events leading up to, but not including, those directly associated with NF-κB-dependent transcription in the nucleus. The "canonical" NF-κB signaling includes the release of active NF-κB in the cytoplasm by the activation of the cytoplasmic kinase complex, known as the IκB kinase (IKK). IKK consists of three major proteins: two kinases, IKKα/IKK1 and IKKβ/IKK2, and a regulatory subunit, IKKγ/NEMO (NF-κB essential modulator). A large body of literature describes the complex mechanisms that are employed to cause IKK activation in response to a wide array of structurally and functionally unrelated extracellular and intracellular signals [11]. In most cases, activated

* Corresponding author.

IKK promotes phosphorylation of IκBα and its subsequent degradation by the ubiquitin-dependent 26S proteasome system, thereby exposing the NLS of NF-κB, leading to its translocation into the nucleus. During its liberation from IκBα and subsequent to nuclear translocation, NF-κB subunits can undergo several posttranslational modifications to acquire maximal capacity to regulate the transcription of many different target genes involved in inflammatory responses, B and T cell receptor activation and various stress responses. The specific outcomes of NF-κB-dependent transcription are dependent on the cell types and the signals leading to NF-κB activation [12]. In addition to this highly studied NF-κB activation mechanism, another well-established "noncanonical" pathway has also emerged, which involves proteolytic processing of p100/NF-κB2 to selectively activate p52/RelB heterodimers. This pathway is solely dependent on IKKα, without the need for IKKβ and NEMO, and regulates secondary lymphoid organogenesis and B cell development, among other biological functions [13]. Readers are encouraged to consult more comprehensive and excellent reviews of the NF-κB family members, distinct activation mechanisms and their role in transcription [2, 3, 12].

It has been demonstrated time and again that differential posttranslational modifications aid in determining the specificity of signal transduction pathways both activating and sometimes inhibiting NF-κB induced by diverse agents. Modifications such as phosphorylation, ubiquitination and acetylation and, more recently, nitrosylation, neddylation and sumoylation have been demonstrated to play critical roles in regulating NF-κB signaling and transcriptional function [14]. One area of NF-κB biology that has not been extensively reviewed in the literature is the role of protein modifications by SUMO (small ubiquitin-like modifier). Thus, the focus of this review will be geared towards current knowledge regarding the roles of sumoylation of different protein targets in NF-κB signaling and transcriptional pathways. We will first briefly describe the process of sumoylation and then delve into different situations in which sumoylation and SUMO regulatory proteins have been implicated in the regulation of NF-κB. Finally, we will highlight some of the unanswered issues that may be further addressed in future research.

Overview of protein sumoylation

Sumoylation is the process of posttranslational covalent modification of target proteins by a relatively small peptide (~20 kDa) called SUMO. SUMO was cloned over 10 years ago in *Saccharomyces cerevisiae* and called *SMT3* (suppressor of mif two 3) [15]. In humans there are currently four known SUMO isoforms (SUMO-1, SUMO-2, SUMO-3 and SUMO-4), of which only SUMO-1, -2, and -3 can be conjugated to target substrate proteins [16]. SUMO is first translated in an unconjugatable precursor state and needs to be C-terminally processed by SUMO proteases to expose glycine-glycine ("di-glycine") residues critical for the conjugation reaction (see below). SUMO-4 contains a proline residue N-terminally adjacent to the di-glycine moiety, which seems to prevent its maturation into a functional conjugatable entity [17]. Protein modification by SUMO occurs on specific lysine residues of target proteins and takes place *via* a series of enzymatic steps. A heterodimeric SUMO-activating enzyme (E1), SAE1/SAE2 in human (also known as Aos1/Uba2 in *S. cerevisiae*), forms a high-energy thioester bond with the active site cysteine residue C173 in SAE2 and the C-terminal glycine residue of SUMO in a manner that is ATP-dependent. The SUMO moiety is then transferred to the catalytic cysteine residue (C93) of Ubc9 (ubiquitin-conjugating enzyme 9), the SUMO-conjugating enzyme (E2). The E2 can then directly interact with a substrate by recognition of a consensus sequence, ψKxE/D ("ψ" representing a hydrophobic amino acid residue and "x" representing any amino acid residue), and transfer SUMO to the epsilon amino group of the lysine residue embedded within the consensus site to form a covalent isopeptide bond; this is referred to as sumoylation [18]. In yeast and mammals, there is only one known SUMO E1 and one known SUMO E2. Both SUMO E1 and E2 are predominantly found in the nuclear compartment, although the E2 has also been shown to be localized to the cytoplasmic filament of the nuclear pore complex (NPC) [19]. Sumoylation affects many protein substrates and is involved in many important physiological and pathological processes [16, 20]. In fact, deletion of Ubc9 has been shown to be lethal in both yeast and mice [21, 22].

Similar to the ubiquitination system [23], sumoylation can utilize a group of enzymes known as SUMO ligases (E3), which can promote the protein sumoylation reaction both *in vitro* and *in vivo*. There are multiple SUMO E3s identified, and the list is still growing. Like E1 and E2, the cellular location of E3s appears to be concentrated in the nucleus or on the nuclear membrane [24]. The largest group of SUMO E3s belongs to the protein inhibitors of activated STAT (PIAS) family, which was originally identified to be inhibitors of the STAT (signal transducer and activator of transcription) family of transcription factors [25, 26]. Characteristic of the PIAS family is their SP-RING (Siz/PIAS-really interesting new gene) domain, which is critical to promote protein

sumoylation [27]. Two additional SUMO E3s, RanBP2 (Ran-binding protein 2) and Pc2 (polycomb group (PcG) protein), have been identified; however, they are structurally dissimilar to the PIAS family as well as to each other [28, 29]. It has been proposed that SUMO E3s may orient the SUMO-bound E2 onto the protein target to increase sumoylation efficiency [30]. SUMO E3s can also provide some substrate specificity; however, the specificity does not appear to be too strict, as certain SUMO E3s exhibit an ability to promote sumoylation of multiple seemingly unrelated substrates both *in vitro* and *in vivo* [24, 31]. Given the relatively small number of E3s currently known compared to the large number of SUMO substrates identified, it seems logical that many substrates share specific E3s or may not even require the aid of an E3 for efficient sumoylation *in vivo*.

Protein sumoylation is a highly dynamic process in the cell, and the detection of sumoylated proteins has proven very difficult with many protein substrates *in vivo* [16]. Limited pools of SUMO precursors and mature SUMO isoforms and the existence of a family of SUMO-specific proteases (SENPs) are most probably the major contributors to the dynamic nature of sumoylation [32]. The SENPs are a group of cysteine proteases that not only participate in maturation of SUMO precursors (often referred to as "processing") but also cleave isopeptide bonds of SUMO-conjugated protein targets (referred to as "desumoylation") [33]. The first SENP was also discovered in yeast and named Ulp (Ubl-specific protease) 1 [34]. Currently, there are 6 human SENPs known (SENP1, 2, 3, 5, 6 and 7). SENP1 is localized in the nucleus [35, 36], and SENP2 is associated with the nuclear envelope [37, 38]. Both SENP3 [39] and SENP5 [40, 41] localize to the nucleolus, and SENP6 [42, 43] and SENP7 [44] also have nuclear distribution. It seems clear that SUMO proteases vary in their ability to both process different SUMO precursors and differentially desumoylate different SUMO isoforms from distinct target substrates [40, 41, 43, 45–47]. Defining substrate and SUMO isoform deconjugation specificities for each of the SUMO proteases is an important current topic of study. In addition, knockout mouse strains missing one of the SUMO E3s and proteases have been generated to provide insights into their physiological roles [48–51]. However, these studies are incomplete at present.

Sumoylation components regulating NF-κB signaling/transcriptional activity

Case 1: The role of IκBα sumoylation

The first SUMO-modified protein implicated in NF-κB regulation is IκBα [52]. This particular NF-κB inhibitor plays multiple critical roles in regulating both initial activation of NF-κB and the duration of this activity in response to extracellular signals. As described below, sumoylation of IκBα could participate at these different steps (Fig. 1). First, degradation of IκBα in response to various cellular stimuli results in initial NF-κB activation [53]. The degradation of IκBα occurs through phosphorylation on serines 32 and 36, which then leads to its recognition by an SCF (Skp1/Cul1/F-box protein) ubiquitin ligase containing β-TrCP (beta-transducin repeat-containing protein). This in turn causes the K48-linked "canonical" polyubiquitination of IκBα predominantly on K21 and/or K22 and results in its proteasome-mediated degradation [54–57]. The SUMO E2 Ubc9 was first shown to interact with IκBα *in vitro*, and overexpression of a catalytically inactive mutant of Ubc9 delayed both IκBα degradation and NF-κB activation induced by TNFα (tumor necrosis factor-alpha) stimulation [58]. In an independent line of investigation, a SUMO-1-modified form of IκBα that was resistant to ubiquitin-mediated degradation was detected in several cell lines [52]. Ubc9 was further shown to function as a SUMO E2 for IκBα. The resistance of sumoylated IκBα to ubiquitin-dependent degradation was found to be the result of targeting of the same lysine residue normally used for ubiquitination, namely K21 [52] . This landmark study demonstrated that competition of sumoylation and ubiquitination for the same lysine can lead to inhibition of proteasomal degradation of a specific protein substrate.

Previous studies demonstrated that ubiquitination of IκBα does not absolutely depend on K21 and can take place on K22 alone to promote proteasome-mediated degradation [56]. It is possible that due to its globular structure, sumoylation of K21 hinders ubiquitination of K22 by the large β-TrCP SCF complex. Since the N-terminal domain of IκBα appears to be quite flexible [4, 5], K21 sumoylation might also induce a conformational change to prevent recognition by the SCF complex. Interestingly, mutation of the IKK phosphorylation sites S32 and S36 to glutamic acid prevented IκBα sumoylation *in vitro* [52]. Thus, a conformational change could also be associated with modulation of IκBα sumoylation by IKK-dependent phosphorylation.

Surprisingly, later studies revealed that amino acids 1–26 of IκBα could be SUMO-modified *in vitro* but

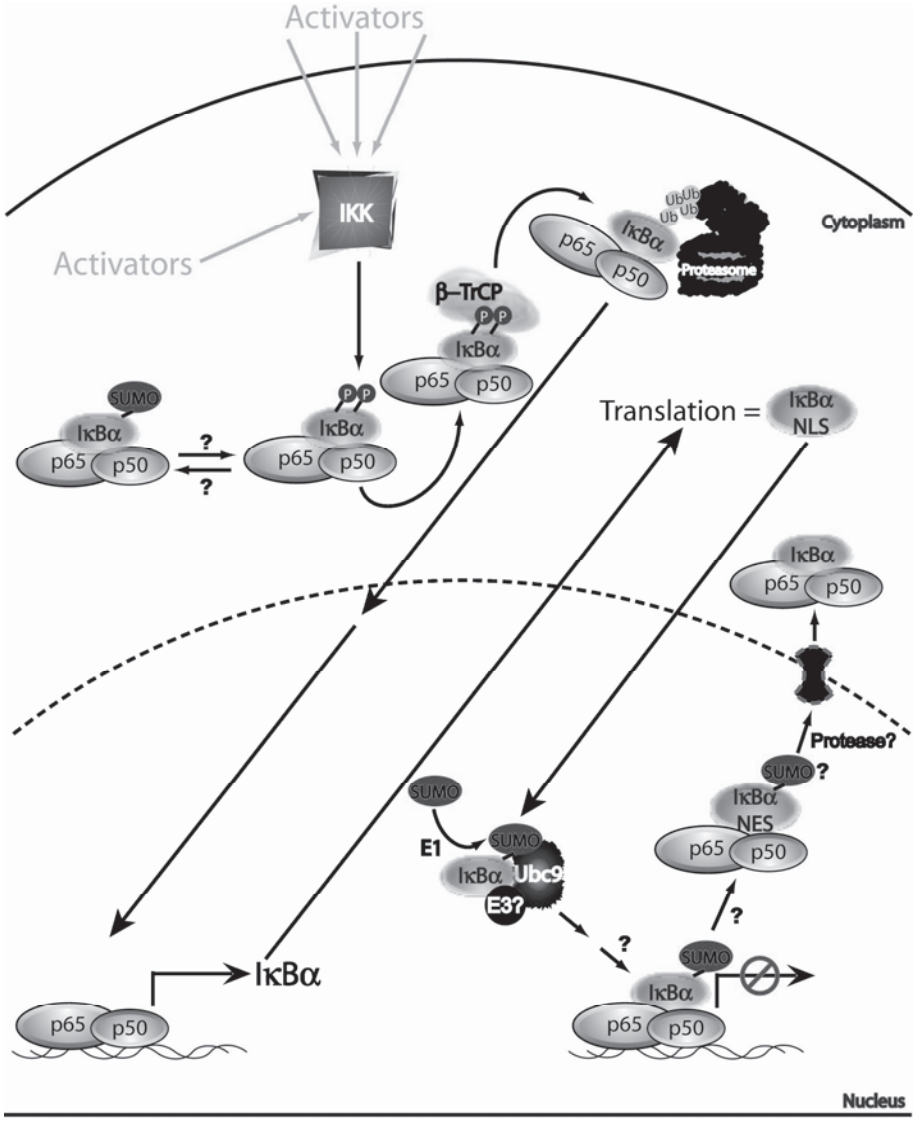

Figure 1. Model showing alternative ways in which sumoylated IκBα could affect the activity of NF-κB. Activators of NF-κB lead to IKK-mediated phosphorylation of IκBα on S32 and S36. This recruits the ubiquitin ligase β-TrCP and results in polyubiquitination of IκBα on K21 and/or K22, leading to its degradation *via* the 26S proteasome. NF-κB then translocates into the nucleus and promotes transcription of its target genes, such as IκBα. Newly translated free IκBα can enter the nucleus *via* its nuclear localization sequence (NLS) and bind NF-κB to inhibit its transcriptional activity. IκBα then brings NF-κB into the cytoplasm *via* its nuclear export sequence (NES), resulting in a cytoplasmic inactive NF-κB/IκBα complex. Sumoylation on K21 has been implicated in inhibition of IκBα degradation, thus providing a mode of NF-κB inhibition. IκBα requires a nuclear signal for its sumoylation that may or may not be enhanced by a SUMO E3. Sumoylated IκBα could play a role in modulating NF-κB-dependent transcription in the nucleus and/or the export of NF-κB from the nucleus. The SUMO protease for IκBα remains unknown. Since most SUMO proteases are localized in the nucleus or at the nuclear periphery, it is possible that an unknown SUMO protease could desumoylate IκBα in conjunction with its exit from the nucleus.

not *in vivo* [59]. Addition of an NLS to IκBα could restore its sumoylation *in vivo*, suggesting that nuclear localization of IκBα was necessary for its sumoylation. Interestingly, free IκBα has been demonstrated to actively transport to the nucleus *via* a nonclassical nuclear localization sequence (NLS) [60]; this nonclassical NLS is located within its second ankyrin repeat and consists of a small cluster of hydrophobic residues [61]. Nuclear localization of free IκBα has an important function in the autoregulatory feedback of NF-κB activity. In a simple yet elegant mechanism, active NF-κB promotes the transcription of IκBα due to an NF-κB responsive element in its promoter [62, 63]. Newly translated IκBα enters the nucleus *via* its unusual NLS and can then "strip" NF-κB off of its DNA element [64, 65] and bring it back to the cytoplasm *via* an IκBα nuclear export sequence (NES). Two NES motifs in IκBα have been identified, one located on its N-terminus and one with a C-terminal location [6–10]. Cell-based analyses support a critical role for both of these NES sequences in the regulation of NF-κB; however, mouse knock-in studies might be necessary to determine the physiological functions of the two sequences. Whether sumoylation of IκBα participates in this elaborate "post-induction repression" process has not been directly evaluated. As sumoylation of IκBα requires its nuclear localization, this may be the case, as previously proposed by others [59, 66]. In this context, the modulatory effects of sumoylation of IκBα on its affinity for NF-κB, subcellular localization, ability to remove NF-κB off DNA sites and nuclear export of NF-κB have not been carefully analyzed. Furthermore, whether or not a SUMO E3 is involved in sumoylation of IκBα and if there is a specific SUMO protease that removes the SUMO moiety from IκBα

have yet to be determined. These additional factors may also change the balance between sumoylated and non-sumoylated pools of IκBα to further fine-tune NF-κB functions.

It was recently described that overexpression of SUMO-4 could modify IκBα and inhibit NF-κB activation in response to TNFα [67]. However, it has yet to be determined if endogenous proteins can be modified by endogenous SUMO-4; the presence of a proline residue near the di-glycine moiety may prevent maturation of SUMO-4 by SUMO proteases [17].

Case 2: Sumoylation of NEMO during NF-κB signaling induced by DNA-damaging agents

The most recently identified SUMO-modified protein substrate in the NF-κB signaling pathway is NEMO [68] (Fig. 2). NEMO, as stated previously, is part of the cytoplasmic IKK complex that is critical for NF-κB activation not only by the majority of extracellular signals, including TNFα and IL-1 (Interleukin-1), but also in response to many genotoxic stress agents, such as etoposide (VP16), camptothecin (CPT) and ionizing radiation (IR) [53, 69]. Structurally, from its N- to C-terminus, NEMO is predicted to contain two putative coiled coil domains (CC1 and CC2), a leucine zipper (LZ) and a zinc finger domain (ZF). NEMO is capable of multimerization; however, the stoichiometry of this multimer appears to be complex *in vivo*, given that dimeric, trimeric and tetrameric forms of NEMO have been described [70–73].

Multimerization of NEMO has been proposed to be critical for activation of the IKK complex, and its multimerization is thought to require the CC2 and LZ trimerization domain on one hand [72] and the N-terminal dimerization domain on the other [73]. Moreover, forced dimerization of NEMO is sufficient to cause IKK and NF-κB activation [70].

The uniqueness of the role of SUMO in genotoxic stress signaling to NF-κB lies upstream of IKK activation events. It was previously demonstrated that enucleation had little impact on NF-κB signaling induced by TNFα, thereby indicating that all the signaling components and events can take place without the need for an intact nucleus. In contrast, the enucleation procedure abrogated activation by CPT, a topoisomerase I poison [74]. This study suggested that a nuclear event, or at least an intact nucleus, is selectively required for genotoxic stress-induced NF-κB activation. Since enucleation leaves mitochondria in the cytoplasts [75], and this abundant organelle contains its own DNA that can also serve as a target for CPT to cause DNA damage *via* the mitochondria-specific topoisomerase I enzyme [76], the study demonstrated that nuclear topoisomerase I is the critical target for the NF-κB activation event to take place. Indeed, CEM/C2 cells that harbor intact mitochondrial topoisomerase I but contain a CPT-resistant nuclear enzyme were defective for NF-κB activation by CPT but not by TNFα or other DNA-damaging agents that do not target topoisomerase I [74]. These and additional studies [77] seem to point to DNA double-strand breaks (DSBs) as the critical

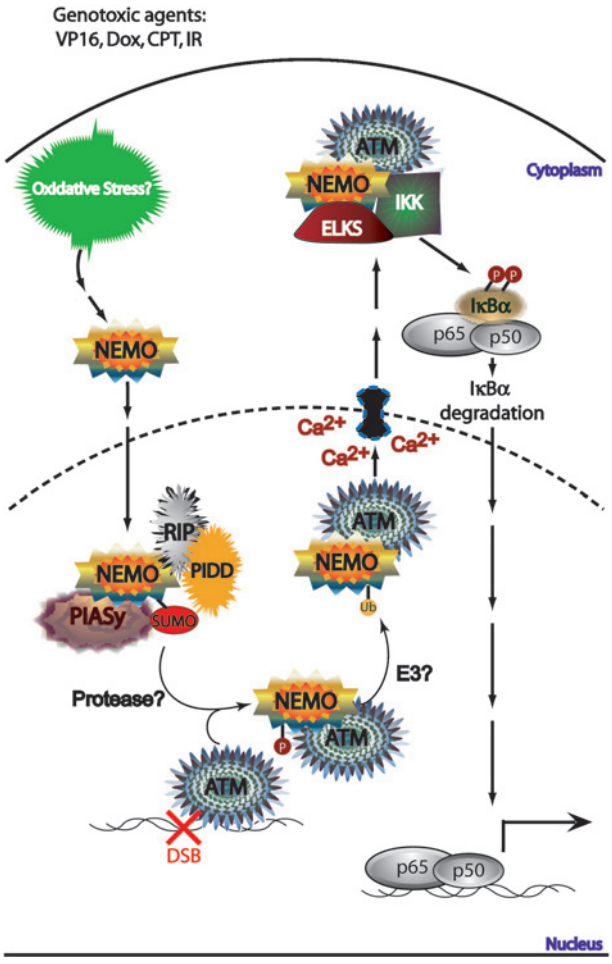

Figure 2. Model depicting the role of sumoylation in genotoxic stress-induced NF-κB signaling. Genotoxic agents cause DNA double-strand breaks (DSBs) along with other cellular stress, including oxidative stress. The latter might be involved in modulating NEMO localization to the nucleus, possibly *via* the C-terminal ZF domain. NEMO is then SUMO-modified on K277/K309 with the help of the SUMO E1/E2 and an E3, PIASy. PIDD and RIP-1 also have a role in NEMO sumoylation. DSBs lead to the activation of ATM, which then associates with NEMO and promotes phosphorylation on S85. This in turn results in NEMO ubiquitination, supposedly on the same lysines that are used for sumoylation. NEMO is exported out of the nucleus likely with ATM, a mechanism that requires Ran-GTP, an unknown nuclear export receptor and Ca^{2+}. Exported ATM and NEMO then work with a protein known as ELKS to promote IKK activation, resulting in activation of NF-κB *via* IκBα degradation. The nature of a SUMO protease, a ubiquitin ligase (E3) and the nuclear export receptor for NEMO and the mechanism of IKK activation remain undetermined.

DNA lesion in promoting NF-κB activation by many genotoxic agents. Later, ataxia telangiectasia mutated (ATM), a nuclear DSB-activated protein kinase, was shown to be essential for NF-κB activation in response to DSB inducers [68, 78]. Although these findings and others [79–81] suggest the requirement for a nuclear signaling event for genotoxic stress-induced NF-κB activation, they did not explain how ATM signaling caused cytoplasmic IKK activation and subsequent NF-κB activation.

A surprising series of events led to a partial understanding of the relationship between NEMO sumoylation and ATM-dependent activation of the IKK complex leading to NF-κB activation. It was demonstrated that a fraction of IKK-free NEMO was SUMO-1-modified in response to CPT and VP16 but not by TNFα and LPS (lipopolysaccharide), and this caused its nuclear accumulation in the cell [68]. This sumoylation was specific for SUMO-1 (not SUMO-2 or -3), since SUMO-2- or SUMO-3-modified NEMO was not observed [82]. SUMO-1 modification of NEMO was induced in a transient manner that occurred prior to cytoplasmic IKK and NF-κB activation. ATM was found to be irrelevant for promoting sumoylation of NEMO by DSB-inducing agents [68]. Moreover, this sumoylation could also be induced by cell stresses that did not induce DSBs or ATM activation [83], thereby further implicating non-ATM-mediated, but some stress-mediated, events in NEMO sumoylation. The putative SUMO-modification sites were determined by mutagenesis studies to be K277 and K309 within and just outside the CC2 domain, respectively [68]. The sumoylation of NEMO also required its ZF motif *in vivo*, and a ZF mutant of NEMO was incapable of being SUMO-modified and could not accumulate in the nucleus upon CPT and VP16 exposure. Following sumoylation, nuclear NEMO interacted with ATM, which phosphorylated serine 85 of NEMO to promote its ubiquitin modification and nuclear export [84]. As mentioned with IκBα above, the sumoylation and ubiquitination sites appeared to compete for the same lysine sites. Mutation of both lysines to alanine prevented ubiquitination even when SUMO-1 was supplied to NEMO as a fusion moiety, a trick that could complement the ZF-dependent sumoylation defect of NEMO [68]. A recent study further demonstrated that a Ca^{2+}-mediated signaling event is also required for NEMO export into the cytoplasm [85]. Cumulation of data led to the model that nuclear export of NEMO permits ATM to form a complex with cytoplasmic IKK, leading to IKK and NF-κB activation by an as-yet poorly defined mechanism [84]. Inhibition of any of these events eliminated genotoxic stress-induced NF-κB activation. Gene fusion studies indicate that SUMO-1 or ubiquitin directly fused to NEMO can bypass the need for these modifications [68, 84], further supporting the critical roles of these events.

The sumoylation of NEMO is believed to be independent of IKK complex formation. Evidence that supports this model include that dissociation of NEMO from the IKK complex by a NEMO-binding peptide did not disrupt its sumoylation. In contrast to NEMO, nuclear translocation of the IKK subunits (IKKα and IKKβ) was not observed prior to NF-κB activation [68]. This suggests that there is a preexisting IKK-free pool of NEMO or some fraction of NEMO that is dissociated from the IKK complex following stress induction to allow for SUMO modification. The requirement of IKK-free NEMO for sumoylation became evident when the SUMO E3 for NEMO was identified. At the time of the study, the known SUMO E3s consisted of the proteins Pc2 and RanBP2, two unrelated SUMO ligases and the PIAS family consisting of PIAS1, PIAS3, PIASxα, PIASxβ and PIASy. Utilizing siRNA towards these known SUMO E3s, a PIAS family SUMO E3, PIASy (also known as PIASγ), was shown to be specifically required for NF-κB activation in response to genotoxic stress [82]. PIASy was demonstrated to promote NEMO sumoylation both *in vitro* and *in vivo* in a manner that was dependent on its catalytic activity (SP-RING domain), and it was also shown to be necessary for NEMO sumoylation in response to genotoxic stimuli without interfering with ATM activation [82]. Significantly, PIASy was only able to interact with IKK-free NEMO. Even when IKKβ and PIASy were overexpressed, NEMO was unable to assemble an IKKβ-NEMO-PIASy complex, demonstrating that the interaction between NEMO-PIASy and NEMO-IKKβ was mutually exclusive; indeed, an overlapping domain of NEMO was required for these interactions. Moreover, the NEMO-PIASy interaction appears to be predominantly nuclear. Interestingly, the NEMO ZF domain was dispensable for both interaction with PIASy *in vivo* and direct SUMO-1 modification *in vitro*. Additionally, sumoylation of NEMO by Ubc9 and PIASy was selective for SUMO-1, since SUMO-2 and SUMO-3 were not conjugated to NEMO *in vitro*. Certain antioxidants prevented NEMO sumoylation induced by VP16 in CEM T leukemic cells [82] and oxidative stress by means of exogenously added hydrogen peroxide was able to cause NEMO sumoylation. These findings led to a model in which cell stress, possibly an oxidative stress-related event, promotes ZF-dependent nuclear import of NEMO and subsequent PIASy-dependent SUMO-1 modification without involving ATM activation.

A recent study further contributed to our understanding of the control of NEMO sumoylation by genotoxic stress conditions. Janssens et al. found that the protein PIDD (p53-inducible death-domain-containing protein) translocates to the nucleus to stabilize/enhance NEMO sumoylation in the cell following genotoxic insult [86]. The increase in SUMO-modified NEMO also correlated with augmented NF-κB activation. It was further shown that RIP1 (receptor-interacting protein 1), previously found to be critical for genotoxic stress-induced NF-κB activation [87], was also required for NEMO sumoylation in conjunction with PIDD. siRNA-mediated silencing of endogenous PIDD attenuated NEMO sumoylation and NF-κB activation, indicating that PIDD is critical for efficient NF-κB activation by genotoxic agents (see Fig. 2 for model). A more recent study demonstrated that PIDD undergoes autocleavage processing to generate PIDD-C and PIDD-CC peptides [88]. Interestingly, PIDD-C promoted NF-κB activation by genotoxic agents, whereas PIDD-CC specifically assembled with RAIDD (RIP-associated ICH-1/CED-3-homologous protein with a death domain) and procaspase-2 to cause apoptosis [88]. It is still unclear how PIDD-C promotes NEMO sumoylation; however, these additional molecular components add to the exciting, yet complex nature of the NEMO sumoylation regulation induced by DNA-damaging agents.

Of note, other roles for PIASy in regulating NF-κB function have recently been observed. Overexpression of PIASy inhibited NF-κB-dependent luciferase reporter activity in both a 291D-GFP mouse keratinocyte cell line in response to a combination of UVB (ultraviolet B) and TNFα in one study [89] and in HEK293 cells in response to overexpressed TRIF (Toll/IL-1 receptor (TIR) domain-containing adaptor inducing IFN-β) [90]. Whether or not PIASy is acting in these pathways through NEMO sumoylation or through an unidentified NF-κB signaling protein has yet to be determined. The requirement of the catalytic activity of PIASy to mediate the inhibitory action has not been determined in these studies, so it also remains a possibility that the effect of PIASy may be mediated by protein-protein interactions without the need for sumoylation *per se*.

Case 3: SUMO, SUMO enzymes and NF-κB-dependent transcription

A. NF-κB transcriptional repression. An instance in which sumoylation components affect NF-κB transactivation activity was shown with a SUMO E3 PIAS family member, PIAS1. In the first scenario, PIAS1 was shown to interact with the NF-κB family member p65 (RelA), and overexpression of PIAS1 led to inhibition of NF-κB-dependent transcription, as shown using an NF-κB luciferase reporter construct [91]. It was further demonstrated that PIAS1 could also inhibit the NF-κB-dependent target genes *IκBα* and *Bfl1*. PIAS1 was believed to exert its effects through prevention of NF-κB DNA-binding activity in the nucleus of the cell, since PIAS1 inhibition of NF-κB occurred after IκBα degradation and p65 nuclear translocation. Overexpression of PIAS1 could inhibit DNA binding of NF-κB *in vitro* and on the *IκBα* promoter *in vivo*. In PIAS1$^{-/-}$ mice, transcription of the NF-κB-dependent genes for the proinflammatory cytokines TNFα and IL-1β was increased upon stimulation with TNFα. These mice also exhibited slightly elevated basal serum levels of TNFα and IL-1β, indicating that PIAS1 functions to suppress NF-κB-dependent transcription [91]. Furthermore, using chromatin immunoprecipitation (ChIP) assays in a PIAS1-deficient cell line, an increase in p65 recruitment to the *IκBα* promoter was seen upon activation of NF-κB with TNFα. Hence, the ability of PIAS1 to prevent NF-κB-dependent transcription *in vivo* correlated with inhibition or displacement of p65 occupancy from the promoter site. Interestingly, PIAS1 was shown to interact with the transactivation domain (TAD) of p65. Thus, in this case, the inhibitory action of PIAS1 on NF-κB-dependent transcription appears to involve direct protein-protein interactions rather than PIAS1-mediated sumoylation of p65.

In the second setting, PIAS1 also surfaced as a regulator of NF-κB-dependent transcription *via* modulating sumoylation of a different transcription factor, namely PPAR-γ (peroxisome proliferator-activated receptor gamma) [92]. PPAR-γ is a nuclear receptor with a role in regulation of adipogenesis [93]. PPAR-γ also has anti-inflammatory properties that are believed to be in part due to its ability to inhibit NF-κB-dependent transcription [94]. A recent study examining the effect of corepressor complexes for NF-κB revealed that NCoR (nuclear hormone receptor co-repressor) and its related factor SMRT (silencing mediator of retinoic acid and thyroid hormone receptors) in complex with HDAC3 (histone deacetylase 3) can serve as a corepressor complex for a subset of NF-κB-dependent target genes, such as the proinflammatory response gene *iNOS* (inducible nitric oxide synthase) [92]. In a yeast 2-hybrid screen, PIAS1 was identified as a binding partner of PPAR-γ. siRNA targeting PIAS1 demonstrated its requirement for LPS-induced PPAR-γ recruitment to the *iNOS* promoter. Treatment with LPS could also promote PPAR-γ sumoylation, and this was required for transrepression of the *iNOS* promoter. These results led to a model in which LPS-induced sumoylation of PPAR-γ mediated by PIAS1 results in PPAR-γ-

dependent recruitment to the NCoR/SMRT corepressor complex on the *iNOS* promoter. Recruitment of this corepressor prevents NF-κB-dependent transactivation. This PIAS1-PPAR-γ-dependent repression of NF-κB target gene transcription might also play a role in the previous report of PIAS1-mediated NF-κB repression [91]. Analysis of this mechanism on multiple promoters of NF-κB-dependent genes would determine how frequently this mechanism is utilized in the cell.

In a separate study, another SUMO E3, PIAS3, was shown to interact with p65 in a yeast 2-hybrid assay, and this interaction was subsequently confirmed using both *in vitro* and *in vivo* assays [95]. In contrast to PIAS1, PIAS3 interacted with the Rel homology domain (RHD) of p65, the domain responsible for NF-κB dimerization and DNA binding. Furthermore, overexpression of PIAS3 could inhibit TNFα- and IL-1β-induced NF-κB-dependent transcription (shown using a 3xκB luciferase reporter), similar to what was seen with PIAS1. Like PIAS1, the effects of PIAS3 were downstream of IκBα degradation, since overexpression of PIAS3 did not alter phosphorylation-dependent degradation of IκBα in response to TNFα stimulation. Unlike PIAS1, PIAS3 did not seem to alter p65 DNA-binding activity. It is well-established that CBP [CREB (cAMP response element-binding)-binding protein] acts as a coactivator of p65 [96, 97]. Interestingly, when overexpressed, PIAS3 could compete with CBP for binding to p65 both *in vitro* and *in vivo* [95]. Hence, the effects of PIAS3 on p65-mediated NF-κB transactivation appear to be due to defects in coactivator binding to p65. Whether or not the catalytic domain within the RING finger of PIAS3, which has been shown to be required for enhanced substrate sumoylation, is required for inhibition of NF-κB transcription has not been determined. This is also the case with PIAS1, described above. Thus, it is still unclear whether the enzymatic activity of PIAS1/3 is required for repression of NF-κB transcriptional activity. Of note, it is of interest that components of the p65 coactivator complex, CBP and p300, are SUMO-modified themselves [98, 99]. SUMO modification of CBP and p300 in this context serves as a negative regulator of transcriptional activity. It is tempting to think that CBP and p300 coactivator regulation by SUMO modification may play a role in negative regulation of NF-κB transcriptional activity. Further studies will need to be conducted to determine if this mechanism indeed exists.

The strong role of sumoylation in NF-κB regulation and possibly NF-κB-dependent transcription was initially identified in genetic studies of innate immunity in the Drosophila system. Mutations in the Drosophila version of the Ubc9 (dUbc9) gene, *lesswright* (*lwr*), were associated with up-regulation of the NF-κB pathway in the fat body, where increased transcriptional activity of Dorsal (dl) and Dif (Dorsal-related immunity factor), members of the Rel/NF-κB family, resulted in constitutive expression of the antimicrobial peptide genes *Drosomycin* and *Cecropin* [100]. Constitutive expression of these genes is abolished in triple (*lwr*, *Dif* and *dl*) mutants [100]. Moreover, the *dUbc9/lwr* had a separate but similar regulatory effect in Drosophila blood cells (hemocytes). *Lwr* mutants exhibited increased proliferation of hemocytes, and some mutant hemocytes showed localization of Dorsal to the nucleus [101]. The overproliferation phenotype could be suppressed in triple (*lwr*, *Dif* and *dl*) mutants [100, 101], but the target genes of Dorsal/Dif causing the overproliferation were not described. Both the fat body- and hemocyte-based phenotypic defects were also rescued by a non-signaling allele of Cactus [100]. These genetic epistatic experiments suggested that dUbc9 either regulates Dorsal and Dif *via* its effects on Cactus or independently regulates Cactus and Dorsal/Dif in the signal transduction pathway. It is possible that direct sumoylation of Dorsal and Dif results in transcriptional repression of target genes such as that of the mammalian transcription factors c-Jun, p53, androgen receptor (AR), Sp3 and Elk-1 (Ets-like kinase 1) [20]. In this context, it is of interest to note that in Cos7 (transformed monkey kidney) cells, overexpression of Ubc9 or SUMO-1 inhibited NF-κB transcriptional activity induced by TNFα, IL-1 and okadaic acid as measured by an NF-κB-dependent HIV-LTR reporter construct [52]. Alternatively, it is also possible that dUbc9 might function in modifying Cactus stability or in regulating Dorsal localization [100]. A previous study indicated that overexpression of catalytically inactive Ubc9 can retard IκBα degradation and NF-κB activation in response to TNFα stimulation in HeLa cells [58].

B. NF-κB transcriptional activation. Although overexpression of dUbc9 was shown to inhibit Dorsal-dependent transcription, as described above, studies examining the effect of dUbc9 on Drosophila development demonstrated the converse [102]. Dorsal was shown to interact with dUbc9 in a yeast 2-hybrid assay, and this interaction was confirmed *in vivo*. Overexpression of Dorsal in cells can result in its nuclear localization by overcoming inhibition imposed by the endogenous pool of the Drosophila homologue of IκB, Cactus. Hence, Cactus needs to be overexpressed along with Dorsal in order to restore Dorsal's cytoplasmic localization. Under conditions in which Dorsal, Cactus and a coactivator (Twist) were coex-

pressed, overexpression of dUbc9 surprisingly resulted in nuclear accumulation of Dorsal and transactivation of a Dorsal-dependent reporter gene. This is inconsistent with the previous study showing that the lesswright mutation causes an increase in nuclear Dorsal accumulation in hemocytes [101]. Coexpression of Dorsal/Twist/Cactus with Drosophila SUMO or the Drosophila SUMO E1, SAE1/2, could also augment the reporter activity, suggesting that components of the SUMO conjugation machinery could stimulate transcription of NF-κB in the fly [102].

Later, Dorsal was shown to be SUMO-modified on K382 in Drosophila S2 cells [103]. Overexpression of dSUMO and dUbc9 could increase Dorsal-dependent reporter activity and expression of the *CecA1* gene. siRNA targeting dUbc9 and dSUMO led to a reduction in *CecA1* gene induction following LPS stimulation of Drosophila S2 cells. Examination of Drosophila first instar larvae that had hypomorphic P-element disruptions in either dUbc9 or dSUMO also revealed defective *CecA1* gene induction upon challenge with LPS. This is in contrast with the study mentioned above, which demonstrated that mutations in lesswright (dUbc9) can increase *CecA1* gene expression in third instar larvae [100]. These apparent contradictory results in Drosophila larvae and cultured cells support the view that individual proteins (or the stoichiometry of proteins in complexes) subject to sumoylation may have different outcomes depending on the cell system used. These observations also underscore the need to characterize how these biochemical differences result in distinct physiological outcomes. The variable effects of SUMO proteins on NF-κB signaling is summarized to emphasize this idea (Table 1).

Overexpression of dSUMO and dUbc9 could activate Dorsal-dependent reporter activity, and mutation of the SUMO modification site on K383 to A or R also increased Dorsal reporter activity, leaving the question as to how sumoylation and mutation of the putative SUMO modification site in Dorsal could lead to the same phenotype. Using two luciferase reporter constructs, one with a single and one with multiple Dorsal binding sites, it was found that mutant Dorsal was only able to increase luciferase activity when multiple binding sites were present. From these results, Bhaskar et al. [103] concluded that the SUMO modification site of *Dorsal* is actually within a synergy control (SC) motif, which has been shown to inhibit transcriptional synergy through recruitment of a synergy attenuator, an SC factor (SCF) [104]. Both SUMO modification and mutagenesis of K383 to A or R presumably prevent the interaction of an unidentified SC factor with DNA-bound Dorsal/Twist, thwarting the attenuation of Dorsal transactivational activity and thus giving rise to the same transcriptional phenotype. This is analogous to the situation in the mammalian system, in which SC motifs have similarity to sumoylation sites; for example, C/EBPalpha and GR (glucocorticoid receptor) can be SUMO-modified within their SC motifs, leading to uncontrolled gene expression [105, 106]. Thus, although sumoylation of transcription factors is generally linked to their transcriptional repression, there are other instances in which SUMO modification of transcription factors can promote their transcriptional activity. Sumoylation of estrogen receptor alpha (ERα) and heat shock factors 1 and 2 (HSF1 and 2) has also been shown to promote their transcription [107–109]; however, unlike the SC factor situations described above, the mechanisms are unclear as to how direct sumoylation of ERα and HSF1/2 could promote their transcriptional activities.

In another yeast 2-hybrid screen, MEKK1 (mitogen-activated protein kinase/ERK kinase kinase-1), a protein positively implicated in NF-κB signaling [110, 111], was found to directly interact with Ubc9 [112]. Further analysis revealed that this interaction requires the catalytic domain and death domain of MEKK1. Overexpression of Ubc9, regardless of its catalytic activity, was shown to promote TNFα-induced NF-κB transcription in an NF-κB-dependent luciferase reporter assay in HeLa cells. This corroborates a previous study showing that a catalytically dead mutant of Ubc9 retards TNFα-induced NF-κB activation [58]. Moreover, when expressed together, Ubc9 and MEKK1 could act in synergy to induce NF-κB activation in response to TNFα stimulation. It was proposed that Ubc9 may be acting as a scaffolding protein with MEKK1 [112]. We also found that depletion of Ubc9 inhibited NF-κB-dependent luciferase reporter activity in response to TNFα in HEK293 cells (Mabb, unpublished observations). In these scenarios, it appears that Ubc9 has some NF-κB signaling function, possibly without involving sumoylation, upstream of NF-κB transcriptional events.

To help sort out the role of SUMO components in NF-κB signaling and modulation of NF-κB-dependent transcription, we attempted to formulate a model as to how sumoylation and SUMO conjugation machinery may play a role in the NF-κB system in response TNFα (Fig. 3). In this hypothetical model, TNFα binds to the TNFR1 (tumor necrosis factor receptor 1) and causes the recruitment of downstream factors, such as TRADD (TNF-R1-associated death domain protein) and RIP1, to the C-terminal DD (death domain) of TNFR1 [113]. Ubc9 may also impinge on the DD of TNFR1 to mediate protein interactions or promote sumoylation of known or as-yet-unidentified additional factors. MEKK1 is also

Table 1. Putative role of SUMO enzymes in the NF-κB pathway.

Cell line/system used	Stimulus	Sumoylation component(s) examined	Experiment	Suggested effects on substrate	Effects on NF-κB	Reference
HeLa	TNFα	Dominant negative Ubc9	Overexpression	Inhibition of IκBα degradation	Inhibition	[58]
HeLa	TNFα	Ubc9 and MEKK1	Overexpression	Interaction with MEKK1	Activation	[112]
HeLa	TNFα	Ubc9	Overexpression	Effects on MEKK1	Activation	[112]
HeLa	TNFα	Dominant negative Ubc9	Overexpression	Effects on MEKK1	Activation	[112]
Cos7	TNFα	SUMO-1	Overexpression	Effects on IκBα sumoylation	Inhibition	[52]
Cos7	IL-1	SUMO-1	Overexpression	Effects on IκBα sumoylation	Inhibition	[52]
Cos7	Okadaic acid	SUMO-1	Overexpression	Effects on IκBα sumoylation	Inhibition	[52]
Cos7	TNFα	Ubc9	Overexpression	Effects on IκBα sumoylation	Inhibition	[52]
Cos7	IL-1	Ubc9	Overexpression	Effects on IκBα sumoylation	Inhibition	[52]
Cos7	Okadaic acid	Ubc9	Overexpression	Effects on IκBα sumoylation	Inhibition	[52]
HEK 293	VP16	Ubc9	Ubc9 siRNA	Effects on NEMO sumoylation	Inhibition	[82]
HEK 293	TNFα	Ubc9	Ubc9 siRNA	Unknown	Inhibition	Mabb, unpublished
HEK 293	TNFα	SUMO-4	Overexpression	Potential IκBα sumoylation	Inhibition	[67]
Drosophila S2	-	Drosophila Ubc9	Overexpression with Dorsal and Twist	Dorsal	Activation	[103]
Drosophila S2	-	Drosophila Ubc9	Overexpression with Dorsal, Cactus, and Twist	Dorsal	Activation	[102]
Drosophila S2	-	Drosophila Ubc9/ SUMO-1	Overexpression with Dorsal	Dorsal	Activation	[103]
Drosophila S2	-	Drosophila Ubc9/ SUMO-1	Overexpression with Dorsal and Twist	Dorsal	Activation	[103]
Drosophila S2	-	Drosophila SUMO	Overexpression with Dorsal, Cactus, and Twist	Dorsal	Activation	[102]
Drosophila S2	-	Drosophila Ubc9/ SUMO-1	Overexpression with Dorsal, Cactus, and Twist	Dorsal	Activation	[102]
Drosophila S2	-	Drosophila Sae1/2 (SUMO E1)	Overexpression with Dorsal, Cactus, and Twist	Dorsal	Activation	[102]
Drosophila S2	-	Drosophila Sae1/2 (SUMO E1)	Overexpression with Dorsal, Cactus, Twist, and Smt3	Dorsal	Activation	[102]
larval hemocytes	-	Drosophila Ubc9	lesswright hypomorphic P-element disruption	Unknown	Dorsal nuclear localization	[101]
larval hemocytes	-	Dominant negative Drosophila Ubc9	Overexpression	Unknown	Dorsal nuclear localization	[101]
HEK293 and CEM	VP16	PIASy	siRNA	NEMO sumoylation	Inhibition	[82] and Mabb, unpublished
HEK293 and CEM	CPT	PIASy	siRNA	NEMO sumoylation	Inhibition	[82] and Mabb, unpublished

Table 1 *(Continued)*

Cell line/system used	Stimulus	Sumoylation component(s) examined	Experiment	Suggested effects on substrate	Effects on NF-κB	Reference
291D-GFP mouse keratinocyte	UVB/ TNFα	PIASy	Overexpression	Unknown	Inhibition	[89]
HEK 293	-	PIASy	Overexpression with TRIF	Unknown	Inhibition	[90]
HEK 293	-	PIASy	Overexpression with TRAF6	Unknown	Inhibition	[90]
MEF	TNFα	PIAS1	PIAS1$^{-/-}$ MEFs	p65	Increase in NF-κB target genes	[91]
HEK 293T and A549	TNFα	PIAS1	Overexpression	p65	Inhibition	[91]
HEK 293T	TNFα	PIAS3	Overexpression	p65	Inhibition	[95]
HEK 293T	IL-1β	PIAS3	Overexpression	p65	Inhibition	[95]
HEK 293T	-	PIAS3	Overexpression with RANK	p65	Inhibition	[95]
HEK 293T	-	PIAS3	Overexpression with TNFR1	p65	Inhibition	[95]
HEK 293T	-	PIAS3	Overexpression with CD30	p65	Inhibition	[95]
HEK 293T	-	PIAS3	Overexpression with TRAF2	p65	Inhibition	[95]
HEK 293T	-	PIAS3	Overexpression with TRAF5	p65	Inhibition	[95]
HEK 293T	-	PIAS3	Overexpression with TRAF6	p65	Inhibition	[95]
MCF-7	TNFα	PIAS3	siRNA	p65	Activation	[95]
HeLa	TNFα	SENP1	Overexpression	Unknown	Inhibition	[117]
HeLa	TNFα	Catalytic defective SENP1	Overexpression	Unknown	Inhibition	[117]
Drosophila S2	-	Drosophila Ulp1	Overexpression with Dorsal and Twist	Dorsal	Biphasic response	[103]

Known SUMO-modified proteins in the NF-κB pathway

Protein	stimulus	Site Required for Modification	Effects of modification on NF-κB signaling	
IκBα	TNFα	K21	Inhibition of IκBα degradation	[52]
NEMO	VP16, CPT	K277/K309	Activation	[68]
NEMO	H2O2	-	Activation	[83]
NEMO	VP16, Dox	-	Activation	[86]
Dorsal	-	K382	Activation?	[103]

activated and interacts with Ubc9 to cause activation of downstream components that promote IKK activation leading to phosphorylation-mediated degradation of IκBα and translocation of NF-κB into the nucleus. Here, Ubc9 may also be involved in the interaction with p50 [58] to either promote or inhibit transcription depending on the cellular context. Upon DNA binding and transcription of NF-κB target genes, PIAS1 may be recruited to a subset of the genes to dissociate NF-κB from its promoter site, perhaps through facilitating SUMO modification and/or displacing bound NF-κB with an unknown inhibitory factor similar to NcoR/SMRT for PIAS1. Alternatively, PIAS3, along with a potential sumoylated corepressor, could be recruited to a subset of NF-κB target genes to prevent recruitment of one of its coactivators, CBP. The signal specificity for PIAS1 or PIAS3 may depend on the context of the promoter. Depending on its positioning inside the cell, the existence of SUMO-modified IκBα may further prevent NF-κB target gene transcription through either sequestration of cytoplasmic NF-κB or sequestration of nuclear NF-κB to prevent target gene transcription.

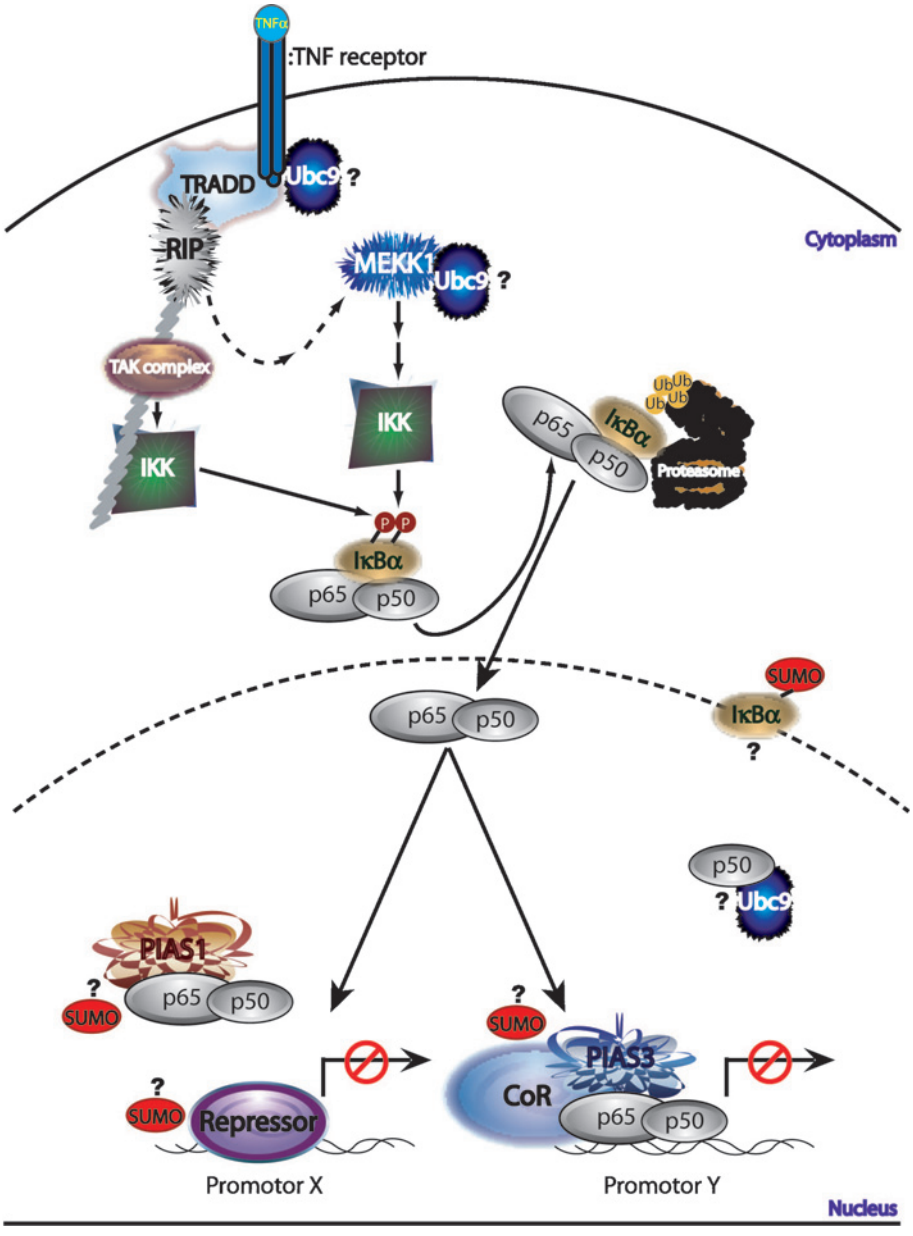

Figure 3. Model depicting possible roles for sumoylation and SUMO enzymes in TNFα-induced NF-κB activation. In response to TNFα treatment, TRADD and RIP are recruited to the death domain of the TNFR. In addition to the signaling pathway that involves non-canonical K63-linked polyubiquitination [125], MEKK1 could also participate in IKK activation. IKK then phosphorylates S32/S36 of IκBα, which promotes its ubiquitination and degradation by the 26S proteasome. Free NF-κB (p65/p50) translocates into the nucleus, where it can promote transcription of its target genes. Attenuation of NF-κB transcription through sumoylation or SUMO enzymes could be achieved in at least two ways: In the first mechanism, the SUMO E3 PIAS1 binds to NF-κB and inhibits its DNA-binding activity. PIAS1 may also be involved in the recruitment of a repressor that could prevent transcription of some NF-κB target genes. In the second mechanism, another SUMO E3, PIAS3, binds to NF-κB to inhibit its transactivation activity; this is most likely achieved through the recruitment of a corepressor complex. Whether or not sumoylation is involved in these two pathways is unclear. The physiological roles of the interactions of Ubc9 with p50, MEKK1 and TNFR1 are also currently undefined.

Perspectives and future studies

The critical roles of sumoylation and SUMO enzymes in NF-κB signaling continue to be elucidated using Drosophila and a variety of mammalian systems. SUMO and the SUMO enzymes seem to participate at the levels of membrane receptor, cytoplasmic signaling components, nuclear signaling components and nuclear transcriptional machinery to modulate NF-κB functions in a manner dependent on cell type and specific signals. Given the existence of many putative sumoylation sites in most NF-κB signaling proteins (see below), it is possible that the list of proteins that become modified by individual SUMO isoforms may expand in the near future. Even in situations in which specific substrates have been identified and their functions partially elucidated, such as IκBα and NEMO, there are many more issues that need to be resolved. For example, specific nuclear roles of SUMO-1-modified IκBα have yet to be defined. It is also uncertain whether IκBα is modified by endogenous SUMO-4. The potential involvement of a SUMO E3 (or E3s) and a protease (or proteases) also needs to be sorted out for this substrate. In the case of NEMO sumoylation, *in vitro* SUMO-1 modification by E1/E2 is extremely inefficient compared to IκBα sumoylation under the same conditions (Mabb, unpublished results). Even in the presence of PIASy, the sumoylation of NEMO is still an inefficient process. One explanation for this could be that the NEMO substrate

Table 2. Frequency of potential sumoylation motifs on proteins involved in NF-κB signalling.

Human	SUMO consensus Lysine sites	SUMO consensus Lysine sites with NDSM	Protein Accession Number
IKKα	109, 146, 479, 624	-	NP_001269
IKKβ	147, 238, 628, 704	704	AAC64675
IKKγ/NEMO	129, 277, 285, 325	129, 277, 325	Q9Y6K9
p65 (RelA)	37	-	AAA36408
RelB	33	33	NP_006500
p105/p50	86, 357, 491, 594	594	AAA36361
p100/p52	90, 298, 332	-	NP_002493
c-Rel	26, 112, 361	112	CAA52954
IkappaBα	21, 38	21, 38	AAA16489
IkappaBβ	187, 235	187	NP_002494
IkappaBε	-	-	NP_004547
IkappaBζ	209, 224	209	NP_113607
bcl-3	-	-	AAA51815
TNFR1	340	-	P19438
TRAF2	27, 313, 477	-	NP_066961
TRAF3	52, 107, 191, 429	191	NP_003291
TRAF5	100, 205, 287, 389, 417	205, 417	NP_001029082
TRAF6	67, 124, 142, 319, 453	142	NP_004611
βTrCP1	101	-	NP_378663
βTrCP2	65	-	NP_003930
MEK1	36, 205	36	NP_002746
JNK	-	-	NP_620637
TRADD	-	-	Q15628
TAB1	-	-	NP_006107
TAB2	329, 562	562	NP_055908
TAK1	547	547	NP_663304
A20	386	-	NP_006281
CYLD	201, 575, 665, 718	201, 575	NP_001035814
CARMA1	9, 177, 231, 237, 255, 726, 937, 1012	9, 177, 237, 255, 937	Q9BXL7
MALT1	262, 303, 702	262	Q9UDY8
Bcl10	110	-	O95999
TRIF	415	415	BAC44839
MYD88	161	-	AAC50954
TIRP/TRAM	36, 58	58	NP_067681
IRAK-1	-	-	NP_001020413
MEKK3	48, 65	48, 65	Q99759
Akt	64, 112, 182, 189, 276	112, 182, 276	NP_001014431
NIK	41, 54, 373, 734	41, 54, 373, 734	NP_003945
RSK1 (p90S6K)	119, 198	119, 198	NP_001006666
CKII	79	79	NP_001886
PIDD	-	-	AAG13461
RIP1	105, 140, 305, 565	140, 305	Q13546
ATM	24, 388, 640, 742, 1323, 1820, 1994, 2302, 2421, 2574, 2687, 2816	388, 640, 2421, 2816	AAB65827
ELKS	10, 195, 202, 439, 444, 472, 488, 579, 643, 706, 707, 712, 748, 885, 927, 962, 1078	195, 439, 444, 472, 579, 643, 706, 707, 1078	Q8IUD2
Proteins with SUMO sites	**37 out of 44**	**27 out of 44**	

Many proteins implicated in NF-κB signaling were analyzed for the existence of consensus SUMO modification motifs (ψKxE/D) using the SUMOsp program [118].
Consensus SUMO sites were then refined based on the NDSM (negatively charged amino acid-dependent sumoylation motif). The NDSM consists of acidic residues, namely aspartic and glutamic acid, flanking the SUMO consensus motif [119]. Out of 44 proteins, 37 contained consensus SUMO motifs and 27 contained SUMO consensus lysine sites with the NDSM.

employed is not in an appropriate state (lacking a modification possibly induced under cell stress conditions, in the wrong conformation or multimeric state, or a combination of these variables). This also highlights our lack of specific knowledge regarding the exact nature of "stress" and stress signaling induced by genotoxic agents to promote NEMO sumoylation. The identification of a SUMO protease and its role in regulating NEMO sumoylation also merits careful analyses. Such studies may also help to clarify how PIDD can act as a switch to promote NEMO sumoylation in the nucleus on one hand (*via* PIDD-C) and cell death through "PIDDosome" formation in the cytoplasm on the other (*via* PIDD-CC).

Although NEMO is currently the best characterized SUMO substrate in the NF-κB signaling system, there

are still many unanswered issues that pertain to this modification. One study indicated that nuclear NEMO could bind to CBP and prevent NF-κB-dependent transcription [114], while in another recent study, nuclear NEMO promoted the activity of the transcription factor hypoxia-inducible Factor 2α (HIF2α) [115]. Whether sumoylation of NEMO participates in modulation of these additional events remains to be investigated. Another relevant question is whether there are physiological signals that require NEMO sumoylation to cause NF-κB activation. In this context, it is also relevant to ask whether sumoylation of NEMO is required for NF-κB activation by genotoxic stimuli in most cell types under physiological settings. Sumoylation of NEMO has been described in a limited number of cell systems in response to different genotoxic stimuli [68, 82, 83, 86]. Thus, whether or not SUMO modification of NEMO and the putative SUMO modification sites (K277 and K309) are physiologically important *in vivo* remains undetermined. While mice deficient in PIASγ, the mouse homologue of human PIASy, have been generated and show no major observable developmental phenotype [50, 51], it is difficult to extract specific roles of NEMO sumoylation from this knockout mouse study due to the involvement of PIASy in regulation of different protein targets and potential compensation by other SUMO E3s. Thus, generation of knock-in mice for K270/302R-NEMO, the murine counterparts of human K277/309R, may be necessary to reveal the physiological role of NEMO sumoylation *in vivo*.

The variability seen with Ubc9 and its effects on NF-κB function currently appears confusing. The discrepancies amongst different model systems may involve both specific and pleiotropic effects of Ubc9 impinging directly on proteins in the NF-κB signaling pathway as well as indirectly on other proteins that are not directly involved in NF-κB signaling systems. Interpretation of results obtained with ectopic expression of Ubc9 and its mutants may be difficult, as this type of manipulation may exhibit global effects on cellular sumoylation processes, given that the levels of SUMO precursors, mature SUMO isoforms and sumoylated substrates seem to be tightly controlled in cells. Thus, additional careful analyses are required to reveal the functional significance of interactions between Ubc9 and substrates such as c-Rel, p50, and IκBα [58] and TNFR1 [112]. Similarly, whether PIAS1/3-mediated regulation of p65 involves sumoylation or is simply due to direct protein-protein interactions without the need for sumoylation remains unclear and requires additional studies; a comparison of catalytically active and inactive PIAS1/3 would be useful. There are examples, such as PIASy-mediated repression of the androgen receptor (AR) activity [116], in which PIAS family members can exert their negative transcriptional effects independent of their catalytic activity.

Although the knowledge of the roles of SUMO isoforms and SUMO enzymes in the regulation of NF-κB signaling and NF-κB transactivation is still incomplete, even less is known about SUMO proteases in NF-κB signaling and transactivation. As stated above, only six SUMO proteases have been identified [44]. In the Drosophila system, Drosophila Ulp1, similar to human SENP1, was shown to desumoylate Dorsal and inhibit Dorsal/Twist activation of a *Dorsal*-dependent reporter [103]. In HeLa cells, SENP1 inhibited TNFα- and p65-induced NF-κB luciferase reporter activity; however, this inhibition appeared to be independent of the catalytic activity of SENP1 [117]. For IκBα and NEMO, nothing is known about the specific involvement of SUMO proteases. Similar to the manipulation of SUMO isoforms and Ubc9, the levels and activities of individual SUMO proteases in cells likely impart a large-scale perturbation in the SUMO system. Thus, experimental setups and interpretation of results from SUMO protease manipulations also require careful consideration before defining a SUMO protease specifically involved at each of the signaling steps.

Since the knowledge of SUMO-modified substrates in the NF-κB signaling system is still very limited, we sought to identify potential SUMO substrates and putative sumoylation sites on known NF-κB signaling proteins by means of sequence analysis. Initially, we evaluated the presence of the consensus SUMO modification sites on known NF-κB signaling proteins through the use of a predictive consensus SUMO site program, SUMOsp (http://bioinformatics.lcd-ustc.org/sumosp/index.php), which predicts putative sumoylation sites within proteins based on previously identified SUMO motifs and the consensus ψKxD/E motif [118]. From this analysis, we identified potential SUMO sites in 37 out of the 44 NF-κB signaling proteins examined (Table 2). It was recently proposed that a potentially more accurate predictor of SUMO modification sites could be the identification of acidic residues flanking the SUMO consensus motif [119]. Using these parameters, we narrowed the number of potential SUMO-modified substrates in the NF-κB signaling system down to 27. It is unclear whether any of these sites are modified by SUMO under any physiological or pathological conditions *in vivo*, but it is noteworthy that so many of the NF-κB signaling proteins possess potential SUMO modification sites. However, this may also be a reflection of a generally high frequency of putative SUMO consensus sites, since when components of another signaling system

(the insulin receptor signaling pathway) were analyzed, a similarly large number of proteins were found to possess such a motif. As detection of sumoylated proteins in cells is difficult, a considerable effort may be needed to determine the whole repertoire of SUMO substrates in the NF-κB signaling system. Given the expanding physiological and pathological roles of both the SUMO system [32, 120, 121] and the NF-κB signaling pathways [122–124], the tie between these two fundamentally important cellular pathways is likely to expand substantially through future studies.

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1 Sen, R. and Baltimore, D. (1986) Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell 46, 705 – 716.
2 Gilmore, T. D. (2006) Introduction to NF-kappaB: players, pathways, perspectives. Oncogene 25. 6680 – 6684.
3 Hoffmann, A., Natoli, G. and Ghosh, G. (2006) Transcriptional regulation *via* the NF-kappaB signaling module. Oncogene. 25, 6706 – 6716.
4 Huxford, T., Huang, D. B., Malek, S. and Ghosh, G. (1998) The crystal structure of the IkappaBalpha/NF-kappaB complex reveals mechanisms of NF-kappaB inactivation. Cell 95, 759 – 770.
5 Jacobs, M. D. and Harrison, S. C. (1998) Structure of an IkappaBalpha/NF-kappaB complex. Cell 95, 749 – 758.
6 Arenzana-Seisdedos, F., Turpin, P., Rodriguez, M., Thomas, D., Hay, R. T., Virelizier, J. L. and Dargemont, C. (1997) Nuclear localization of I kappa B alpha promotes active transport of NF-kappa B from the nucleus to the cytoplasm. J. Cell Sci. 110, 369 – 378.
7 Johnson, C., Van Antwerp, D. and Hope, T. J. (1999) An N-terminal nuclear export signal is required for the nucleocytoplasmic shuttling of IkappaBalpha. EMBO J. 18, 6682 – 6693.
8 Sachdev, S. and Hannink, M. (1998) Loss of IkappaB alpha-mediated control over nuclear import and DNA binding enables oncogenic activation of c-Rel. Mol. Cell. Biol. 18, 5445 – 5456.
9 Huang, T. T., Kudo, N., Yoshida, M. and Miyamoto, S. (2000) A nuclear export signal in the N-terminal regulatory domain of IkappaBalpha controls cytoplasmic localization of inactive NF-kappaB/IkappaBalpha complexes. Proc. Natl. Acad. Sci. USA 97, 1014 – 1019.
10 Tam, W. F., Lee, L. H., Davis, L. and Sen, R. (2000) Cytoplasmic sequestration of rel proteins by IkappaBalpha requires CRM1-dependent nuclear export. Mol. Cell. Biol. 20, 2269 – 2284.
11 Scheidereit, C. (2006) IkappaB kinase complexes: gateways to NF-kappaB activation and transcription. Oncogene 25, 6685 – 6705.
12 Perkins, N. D. (2007) Integrating cell-signalling pathways with NF-kappaB and IKK function. Nat. Rev. Mol. Cell. Biol. 8, 49 – 62.
13 Bonizzi, G. and Karin, M. (2004) The two NF-kappaB activation pathways and their role in innate and adaptive immunity. Trends Immunol. 25, 280 – 288.
14 Perkins, N. D. (2006) Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. Oncogene 25, 6717 – 6730.
15 Meluh, P. B. and Koshland, D. (1995) Evidence that the MIF2 gene of *Saccharomyces cerevisiae* encodes a centromere protein with homology to the mammalian centromere protein CENP-C. Mol. Biol. Cell. 6, 793 – 807.
16 Hay, R. T. (2005) SUMO: a history of modification. Mol. Cell 18, 1 – 12.
17 Owerbach, D., McKay, E. M., Yeh, E. T., Gabbay, K. H. and Bohren, K. M. (2005) A proline-90 residue unique to SUMO-4 prevents maturation and sumoylation. Biochem. Biophys. Res. Commun. 337, 517 – 520.
18 Kim, K. I., Baek, S. H. and Chung, C. H. (2002) Versatile protein tag, SUMO: its enzymology and biological function. J. Cell. Physiol. 191, 257 – 268.
19 Melchior, F. (2000) SUMO – nonclassical ubiquitin. Annu. Rev. Cell. Dev. Biol. 16, 591 – 626.
20 Gill, G. (2004) SUMO and ubiquitin in the nucleus: different functions, similar mechanisms? Genes Dev. 18, 2046 – 2059.
21 Nacerddine, K., Lehembre, F., Bhaumik, M., Artus, J., Cohen-Tannoudji, M., Babinet, C., Pandolfi, P. P. and Dejean, A. (2005) The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. Dev. Cell 9, 769 – 779.
22 Seufert, W., Futcher, B. and Jentsch, S. (1995) Role of a ubiquitin-conjugating enzyme in degradation of S- and M-phase cyclins. Nature 373, 78 – 81.
23 Pickart, C. M. (2001) Mechanisms underlying ubiquitination. Annu. Rev. Biochem. 70, 503 – 533.
24 Melchior, F., Schergaut, M. and Pichler, A. (2003) SUMO: ligases, isopeptidases and nuclear pores. Trends Biochem. Sci. 28, 612 – 618.
25 Chung, C. D., Liao, J., Liu, B., Rao, X., Jay, P., Berta, P. and Shuai, K. (1997) Specific inhibition of Stat3 signal transduction by PIAS3. Science 278, 1803 – 1805.
26 Liu, B., Liao, J., Rao, X., Kushner, S. A., Chung, C. D., Chang, D. D. and Shuai, K. (1998) Inhibition of Stat1-mediated gene activation by PIAS1. Proc. Natl. Acad. Sci. USA 95, 10626 – 10631.
27 Hochstrasser, M. (2001) SP-RING for SUMO: new functions bloom for a ubiquitin-like protein. Cell 107, 5 – 8.
28 Pichler, A., Gast, A., Seeler, J. S., Dejean, A. and Melchior, F. (2002) The nucleoporin RanBP2 has SUMO1 E3 ligase activity. Cell 108, 109 – 120.
29 Kagey, M. H., Melhuish, T. A. and Wotton, D. (2003) The polycomb protein Pc2 is a SUMO E3. Cell 113, 127 – 137.
30 Reverter, D. and Lima, C. D. (2005) Insights into E3 ligase activity revealed by a SUMO-RanGAP1-Ubc9-Nup358 complex. Nature 435, 687 – 692.
31 Schmidt, D. and Muller, S. (2002) Members of the PIAS family act as SUMO ligases for c-Jun and p53 and repress p53 activity. Proc. Natl. Acad. Sci. USA 99, 2872 – 2877.
32 Muller, S., Ledl, A. and Schmidt, D. (2004) SUMO: a regulator of gene expression and genome integrity. Oncogene 23, 1998 – 2008.
33 Yeh, E. T., Gong, L. and Kamitani, T. (2000) Ubiquitin-like proteins: new wines in new bottles. Gene 248, 1 – 14.
34 Li, S. J. and Hochstrasser, M. (1999) A new protease required for cell-cycle progression in yeast. Nature 398, 246 – 251.
35 Gong, L., Millas, S., Maul, G. G. and Yeh, E. T. (2000) Differential regulation of sentrinized proteins by a novel sentrin-specific protease. J. Biol. Chem. 275, 3355 – 3359.
36 Bailey, D. and O'Hare, P. (2002) Herpes simplex virus 1 ICP0 co-localizes with a SUMO-specific protease. J. Gen. Virol. 83, 2951 – 2964.
37 Zhang, H., Saitoh, H. and Matunis, M. J. (2002) Enzymes of the SUMO modification pathway localize to filaments of the nuclear pore complex. Mol. Cell. Biol. 22, 6498 – 6508.
38 Hang, J. and Dasso, M. (2002) Association of the human SUMO-1 protease SENP2 with the nuclear pore. J. Biol. Chem. 277, 19961 – 19966.

39 Nishida, T., Tanaka, H. and Yasuda, H. (2000) A novel mammalian Smt3-specific isopeptidase 1 (SMT3IP1) localized in the nucleolus at interphase. Eur. J. Biochem. 267, 6423 – 6427.

40 Gong, L. and Yeh, E. T. (2006) Characterization of a family of nucleolar SUMO-specific proteases with preference for SUMO-2 or SUMO-3. J. Biol. Chem. 281, 15869 – 15877.

41 Di Bacco, A., Ouyang, J., Lee, H. Y., Catic, A., Ploegh, H. and Gill, G. (2006) The SUMO-specific protease SENP5 is required for cell division. Mol. Cell. Biol. 26, 4489 – 4498.

42 Kim, K. I., Baek, S. H., Jeon, Y. J., Nishimori, S., Suzuki, T., Uchida, S., Shimbara, N., Saitoh, H., Tanaka, K. and Chung, C. H. (2000) A new SUMO-1-specific protease, SUSP1, that is highly expressed in reproductive organs. J. Biol. Chem. 275, 14102 – 14106.

43 Mukhopadhyay, D., Ayaydin, F., Kolli, N., Tan, S. H., Anan, T., Kametaka, A., Azuma, Y., Wilkinson, K. D. and Dasso, M. (2006) SUSP1 antagonizes formation of highly SUMO2/3-conjugated species. J. Cell Biol. 174, 939 – 949.

44 Cheng, J., Bawa, T., Lee, P., Gong, L. and Yeh, E. T. (2006) Role of desumoylation in the development of prostate cancer. Neoplasia 8, 667 – 676.

45 Shen, L. N., Dong, C., Liu, H., Naismith, J. H. and Hay, R. T. (2006) The structure of SENP1-SUMO-2 complex suggests a structural basis for discrimination between SUMO paralogues during processing. Biochem. J. 397, 279 – 288.

46 Reverter, D. and Lima, C. D. (2006) Structural basis for SENP2 protease interactions with SUMO precursors and conjugated substrates. Nat. Struct. Mol. Biol. 13, 1060 – 1068.

47 Reverter, D. and Lima, C. D. (2004) A basis for SUMO protease specificity provided by analysis of human Senp2 and a Senp2-SUMO complex. Structure 12, 1519 – 1531.

48 Yamaguchi, T., Sharma, P., Athanasiou, M., Kumar, A., Yamada, S. and Kuehn, M. R. (2005) Mutation of SENP1/SuPr-2 reveals an essential role for desumoylation in mouse development. Mol. Cell. Biol. 25, 5171 – 5182.

49 Liu, B., Mink, S., Wong, K. A., Stein, N., Getman, C., Dempsey, P. W., Wu, H. and Shuai, K. (2004) PIAS1 selectively inhibits interferon-inducible genes and is important in innate immunity. Nat. Immunol. 5, 891 – 898.

50 Roth, W., Sustmann, C., Kieslinger, M., Gilmozzi, A., Irmer, D., Kremmer, E., Turck, C. and Grosschedl, R. (2004) PIASy-deficient mice display modest defects in IFN and Wnt signaling. J. Immunol. 173, 6189 – 6199.

51 Wong, K. A., Kim, R., Christofk, H., Gao, J., Lawson, G. and Wu, H. (2004) Protein inhibitor of activated STAT Y (PIASy) and a splice variant lacking exon 6 enhance sumoylation but are not essential for embryogenesis and adult life. Mol. Cell. Biol. 24, 5577 – 5586.

52 Desterro, J. M., Rodriguez, M. S. and Hay, R. T. (1998) SUMO-1 modification of IkappaBalpha inhibits NF-kappaB activation. Mol. Cell 2, 233 – 239.

53 Hayden, M. S. and Ghosh, S. (2004) Signaling to NF-kappaB. Genes Dev. 18, 2195 – 2224.

54 Chen, Z., Hagler, J., Palombella, V. J., Melandri, F., Scherer, D., Ballard, D. and Maniatis, T. (1995) Signal-induced site-specific phosphorylation targets I kappa B alpha to the ubiquitin-proteasome pathway. Genes Dev. 9, 1586 – 1597.

55 Spencer, E., Jiang, J. and Chen, Z. J. (1999) Signal-induced ubiquitination of IkappaBalpha by the F-box protein Slimb/beta-TrCP. Genes Dev. 13, 284 – 294.

56 Scherer, D. C., Brockman, J. A., Chen, Z., Maniatis, T. and Ballard, D. W. (1995) Signal-induced degradation of I kappa B alpha requires site-specific ubiquitination. Proc. Natl. Acad. Sci. USA 92, 11259 – 11263.

57 Palombella, V. J., Rando, O. J., Goldberg, A. L. and Maniatis, T. (1994) The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. Cell 78, 773 – 785.

58 Tashiro, K., Pando, M. P., Kanegae, Y., Wamsley, P. M., Inoue, S. and Verma, I. M. (1997) Direct involvement of the ubiquitin-conjugating enzyme Ubc9/Hus5 in the degradation of IkappaBalpha. Proc. Natl. Acad. Sci. USA 94, 7862 – 7867.

59 Rodriguez, M. S., Dargemont, C. and Hay, R. T. (2001) SUMO-1 conjugation *in vivo* requires both a consensus modification motif and nuclear targeting. J. Biol. Chem. 276, 12654 – 12659.

60 Turpin, P., Hay, R. T. and Dargemont, C. (1999) Characterization of IkappaBalpha nuclear import pathway. J. Biol. Chem. 274, 6804 – 6812.

61 Sachdev, S., Hoffmann, A. and Hannink, M. (1998) Nuclear localization of IkappaB alpha is mediated by the second ankyrin repeat: the IkappaB alpha ankyrin repeats define a novel class of cis-acting nuclear import sequences. Mol. Cell. Biol. 18, 2524 – 2534.

62 Sun, S. C., Ganchi, P. A., Ballard, D. W. and Greene, W. C. (1993) NF-kappa B controls expression of inhibitor I kappa B alpha: evidence for an inducible autoregulatory pathway. Science 259, 1912 – 1915.

63 Chiao, P. J., Miyamoto, S. and Verma, I. M. (1994) Autoregulation of I kappa B alpha activity. Proc. Natl. Acad. Sci. USA 91, 28 – 32.

64 Zabel, U. and Baeuerle, P. A. (1990) Purified human I kappa B can rapidly dissociate the complex of the NF-kappa B transcription factor with its cognate DNA. Cell 61, 255 – 265.

65 Arenzana-Seisdedos, F., Thompson, J., Rodriguez, M. S., Bachelerie, F., Thomas, D. and Hay, R. T. (1995) Inducible nuclear expression of newly synthesized I kappa B alpha negatively regulates DNA-binding and transcriptional activities of NF-kappa B. Mol. Cell. Biol. 15, 2689 – 2696.

66 Kracklauer, M. P. and Schmidt, C. (2003) At the crossroads of SUMO and NF-kappaB. Mol. Cancer 2, 39.

67 Guo, D., Li, M., Zhang, Y., Yang, P., Eckenrode, S., Hopkins, D., Zheng, W., Purohit, S., Podolsky, R. H., Muir, A., Wang, J., Dong, Z., Brusko, T., Atkinson, M., Pozzilli, P., Zeidler, A., Raffel, L. J., Jacob, C. O., Park, Y., Serrano-Rios, M., Larrad, M. T., Zhang, Z., Garchon, H. J., Bach, J. F., Rotter, J. I., She, J. X. and Wang, C. Y. (2004) A functional variant of SUMO4, a new I kappa B alpha modifier, is associated with type 1 diabetes. Nat. Genet. 36, 837 – 841.

68 Huang, T. T., Wuerzberger-Davis, S. M., Wu, Z. H. and Miyamoto, S. (2003) Sequential modification of NEMO/IKKgamma by SUMO-1 and ubiquitin mediates NF-kappaB activation by genotoxic stress. Cell 115, 565 – 576.

69 Janssens, S. and Tschopp, J. (2006) Signals from within: the DNA-damage-induced NF-kappaB response. Cell Death Differ. 13, 773 – 784.

70 Poyet, J. L., Srinivasula, S. M., Lin, J. H., Fernandes-Alnemri, T., Yamaoka, S., Tsichlis, P. N. and Alnemri, E. S. (2000) Activation of the Ikappa B kinases by RIP *via* IKKgamma / NEMO-mediated oligomerization. J. Biol. Chem. 275, 37966 – 37977.

71 Tegethoff, S., Behlke, J. and Scheidereit, C. (2003) Tetrameric oligomerization of IkappaB kinase gamma (IKKgamma) is obligatory for IKK complex activity and NF-kappaB activation. Mol. Cell. Biol. 23, 2029 – 2041.

72 Agou, F., Traincard, F., Vinolo, E., Courtois, G., Yamaoka, S., Israel, A. and Veron, M. (2004) The trimerization domain of NEMO is composed of the interacting C-terminal CC2 and LZ coiled-coil subdomains. J. Biol. Chem. 279, 27861 – 27869.

73 Marienfeld, R. B., Palkowitsch, L. and Ghosh, S. (2006) Dimerization of the IkappaB kinase-binding domain of NEMO is required for tumor necrosis factor alpha-induced NF-kappaB activity. Mol. Cell. Biol. 26, 9209 – 9219.

74 Huang, T. T., Wuerzberger-Davis, S. M., Seufzer, B. J., Shumway, S. D., Kurama, T., Boothman, D. A. and Miyamoto, S. (2000) NF-kappaB activation by camptothecin. A linkage between nuclear DNA damage and cytoplasmic signaling events. J. Biol. Chem. 275, 9501 – 9509.

75 Wise, G. E. and Prescott, D. M. (1973) Ultrastructure of enucleated mammalian cells in culture. Exp. Cell Res. 81, 63 – 72.

76 Zhang, H., Barcelo, J. M., Lee, B., Kohlhagen, G., Zimonjic, D. B., Popescu, N. C. and Pommier, Y. (2001) Human mitochondrial topoisomerase I. Proc. Natl. Acad. Sci. USA 98, 10608 – 10613.

77 Piret, B. and Piette, J. (1996) Topoisomerase poisons activate the transcription factor NF-kappaB in ACH-2 and CEM cells. Nucleic Acids Res. 24, 4242 – 4248.

78 Li, N., Banin, S., Ouyang, H., Li, G. C., Courtois, G., Shiloh, Y., Karin, M. and Rotman, G. (2001) ATM is required for IkappaB kinase (IKKk) activation in response to DNA double strand breaks. J. Biol. Chem. 276, 8898 – 8903.

79 Piret, B., Schoonbroodt, S. and Piette, J. (1999) The ATM protein is required for sustained activation of NF-kappaB following DNA damage. Oncogene 18, 2261 – 2271.

80 Jung, M., Zhang, Y., Lee, S. and Dritschilo, A. (1995) Correction of radiation sensitivity in ataxia telangiectasia cells by a truncated I kappa B-alpha. Science 268, 1619 – 1621.

81 Lee, S. J., Dimtchev, A., Lavin, M. F., Dritschilo, A. and Jung, M. (1998) A novel ionizing radiation-induced signaling pathway that activates the transcription factor NF-kappaB. Oncogene 17, 1821 – 1826.

82 Mabb, A. M., Wuerzberger-Davis, S. M. and Miyamoto, S. (2006) PIASy mediates NEMO sumoylation and NF-kappaB activation in response to genotoxic stress. Nat. Cell Biol. 8, 986 – 993.

83 Wuerzberger-Davis, S. M., Nakamura, Y., Seufzer, B. J. and Miyamoto, S. (2006) NF-kappaB activation by combinations of NEMO SUMOylation and ATM activation stresses in the absence of DNA damage. Oncogene 26, 641 – 651.

84 Wu, Z. H., Shi, Y., Tibbetts, R. S. and Miyamoto, S. (2006) Molecular linkage between the kinase ATM and NF-kappaB signaling in response to genotoxic stimuli. Science 311, 1141 – 1146.

85 Berchtold, C. M., Wu, Z. H., Huang, T. T. and Miyamoto, S. (2007) Calcium-dependent regulation of NEMO nuclear export in response to genotoxic stimuli. Mol. Cell. Biol. 27, 497 – 509.

86 Janssens, S., Tinel, A., Lippens, S. and Tschopp, J. (2005) PIDD mediates NF-kappaB activation in response to DNA damage. Cell 123, 1079 – 1092.

87 Hur, G. M., Lewis, J., Yang, Q., Lin, Y., Nakano, H., Nedospasov, S. and Liu, Z. G. (2003) The death domain kinase RIP has an essential role in DNA damage-induced NF-kappa B activation. Genes Dev. 17, 873 – 882.

88 Tinel, A., Janssens, S., Lippens, S., Cuenin, S., Logette, E., Jaccard, B., Quadroni, M. and Tschopp, J. (2006) Autoproteolysis of PIDD marks the bifurcation between pro-death caspase-2 and pro-survival NF-kappaB pathway. EMBO J. 26, 197 – 208.

89 Albor, A., El-Hizawi, S., Horn, E. J., Laederich, M., Frosk, P., Wrogemann, K. and Kulesz-Martin, M. (2006) The interaction of Piasy with Trim32, an E3-ubiquitin ligase mutated in limb-girdle muscular dystrophy type 2H, promotes Piasy degradation and regulates UVB-induced keratinocyte apoptosis through NFkappaB. J. Biol. Chem. 281, 25850 – 25866.

90 Zhang, J., Xu, L. G., Han, K. J., Wei, X. and Shu, H. B. (2004) PIASy represses TRIF-induced ISRE and NF-kappaB activation but not apoptosis. FEBS Lett. 570, 97 – 101.

91 Liu, B., Yang, R., Wong, K. A., Getman, C., Stein, N., Teitell, M. A., Cheng, G., Wu, H. and Shuai, K. (2005) Negative regulation of NF-kappaB signaling by PIAS1. Mol. Cell. Biol. 25, 1113 – 1123.

92 Pascual, G., Fong, A. L., Ogawa, S., Gamliel, A., Li, A. C., Perissi, V., Rose, D. W., Willson, T. M., Rosenfeld, M. G. and Glass, C. K. (2005) A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. Nature 437, 759 – 763.

93 Lazar, M. A. (2005) PPAR gamma, 10 years later. Biochimie 87, 9 – 13.

94 Chung, S. W., Kang, B. Y., Kim, S. H., Pak, Y. K., Cho, D., Trinchieri, G. and Kim, T. S. (2000) Oxidized low density lipoprotein inhibits interleukin-12 production in lipopolysaccharide-activated mouse macrophages *via* direct interactions between peroxisome proliferator-activated receptor-gamma and nuclear factor-kappa B. J. Biol. Chem. 275, 32681 – 32687.

95 Jang, H. D., Yoon, K., Shin, Y. J., Kim, J. and Lee, S. Y. (2004) PIAS3 suppresses NF-kappaB-mediated transcription by interacting with the p65/RelA subunit. J. Biol. Chem. 279, 24873 – 24880.

96 Perkins, N. D., Felzien, L. K., Betts, J. C., Leung, K., Beach, D. H. and Nabel, G. J. (1997) Regulation of NF-kappaB by cyclin-dependent kinases associated with the p300 coactivator. Science 275, 523 – 527.

97 Gerritsen, M. E., Williams, A. J., Neish, A. S., Moore, S., Shi, Y. and Collins, T. (1997) CREB-binding protein/p300 are transcriptional coactivators of p65. Proc. Natl. Acad. Sci. USA 94, 2927 – 2932.

98 Kuo, H. Y., Chang, C. C., Jeng, J. C., Hu, H. M., Lin, D. Y., Maul, G. G., Kwok, R. P. and Shih, H. M. (2005) SUMO modification negatively modulates the transcriptional activity of CREB-binding protein *via* the recruitment of Daxx. Proc. Natl. Acad. Sci. USA 102, 16973 – 16978.

99 Girdwood, D., Bumpass, D., Vaughan, O. A., Thain, A., Anderson, L. A., Snowden, A. W., Garcia-Wilson, E., Perkins, N. D. and Hay, R. T. (2003) P300 transcriptional repression is mediated by SUMO modification. Mol. Cell 11, 1043 – 1054.

100 Chiu, H., Ring, B. C., Sorrentino, R. P., Kalamarz, M., Garza, D. and Govind, S. (2005) dUbc9 negatively regulates the Toll-NF-kappa B pathways in larval hematopoiesis and drosomycin activation in Drosophila. Dev. Biol. 288, 60 – 72.

101 Huang, L., Ohsako, S. and Tanda, S. (2005) The lesswright mutation activates Rel-related proteins, leading to overproduction of larval hemocytes in *Drosophila melanogaster*. Dev. Biol. 280, 407 – 420.

102 Bhaskar, V., Valentine, S. A. and Courey, A. J. (2000) A functional interaction between dorsal and components of the Smt3 conjugation machinery. J. Biol. Chem. 275, 4033 – 4040.

103 Bhaskar, V., Smith, M. and Courey, A. J. (2002) Conjugation of Smt3 to dorsal may potentiate the Drosophila immune response. Mol. Cell. Biol. 22, 492 – 504.

104 Iniguez-Lluhi, J. A. and Pearce, D. (2000) A common motif within the negative regulatory regions of multiple factors inhibits their transcriptional synergy. Mol. Cell. Biol. 20, 6040 – 6050.

105 Subramanian, L., Benson, M. D. and Iniguez-Lluhi, J. A. (2003) A synergy control motif within the attenuator domain of CCAAT/enhancer-binding protein alpha inhibits transcriptional synergy through its PIASy-enhanced modification by SUMO-1 or SUMO-3. J. Biol. Chem. 278, 9134 – 9141.

106 Holmstrom, S., Van Antwerp, M. E. and Iniguez-Lluhi, J. A. (2003) Direct and distinguishable inhibitory roles for SUMO isoforms in the control of transcriptional synergy. Proc. Natl. Acad. Sci. USA 100, 15758 – 15763.

107 Sentis, S., Le Romancer, M., Bianchin, C., Rostan, M. C. and Corbo, L. (2005) Sumoylation of the estrogen receptor alpha hinge region regulates its transcriptional activity. Mol. Endocrinol. 19, 2671 – 2684.

108 Hong, Y., Rogers, R., Matunis, M. J., Mayhew, C. N., Goodson, M. L., Park-Sarge, O. K. and Sarge, K. D. (2001) Regulation of heat shock transcription factor 1 by stress-induced SUMO-1 modification. J. Biol. Chem. 276, 40263 – 40267.

109 Goodson, M. L., Hong, Y., Rogers, R., Matunis, M. J., Park-Sarge, O. K. and Sarge, K. D. (2001) Sumo-1 modification regulates the DNA binding activity of heat shock transcription factor 2, a promyelocytic leukemia nuclear body associated transcription factor. J. Biol. Chem. 276, 18513 – 18518.

110 Meyer, C. F., Wang, X., Chang, C., Templeton, D. and Tan, T. H. (1996) Interaction between c-Rel and the mitogen-activated protein kinase kinase kinase 1 signaling cascade in

mediating kappaB enhancer activation. J. Biol. Chem. 271, 8971 – 8976.
111 Lee, F. S., Hagler, J., Chen, Z. J. and Maniatis, T. (1997) Activation of the IkappaB alpha kinase complex by MEKK1, a kinase of the JNK pathway. Cell 88, 213 – 222.
112 Saltzman, A., Searfoss, G., Marcireau, C., Stone, M., Ressner, R., Munro, R., Franks, C., D'Alonzo, J., Tocque, B., Jaye, M. and Ivashchenko, Y. (1998) hUBC9 associates with MEKK1 and type I TNF-alpha receptor and stimulates NFkappaB activity. FEBS Lett. 425, 431 – 435.
113 Chen, G. and Goeddel, D. V. (2002) TNF-R1 signaling: a beautiful pathway. Science 296, 1634 – 1635.
114 Verma, U. N., Yamamoto, Y., Prajapati, S. and Gaynor, R. B. (2004) Nuclear role of I kappa B Kinase-gamma/NF-kappa B essential modulator (IKK gamma/NEMO) in NF-kappa B-dependent gene expression. J. Biol. Chem. 279, 3509 – 3515.
115 Bracken, C. P., Whitelaw, M. L. and Peet, D. J. (2005) Activity of hypoxia-inducible factor 2alpha is regulated by association with the NF-kappaB essential modulator. J. Biol. Chem. 280, 14240 – 14251.
116 Gross, M., Yang, R., Top, I., Gasper, C. and Shuai, K. (2004) PIASy-mediated repression of the androgen receptor is independent of sumoylation. Oncogene 23, 3059 – 3066.
117 Gao, F., Cheng, J., Shi, T. and Yeh, E. T. (2006) Neddylation of a breast cancer-associated protein recruits a class III histone deacetylase that represses NFkappaB-dependent transcription. Nat. Cell Biol. 8, 1171 – 1177.
118 Xue, Y., Zhou, F., Fu, C., Xu, Y. and Yao, X. (2006) SUMOsp: a web server for sumoylation site prediction. Nucleic Acids Res. 34, W254 – W257.
119 Yang, S. H., Galanis, A., Witty, J. and Sharrocks, A. D. (2006) An extended consensus motif enhances the specificity of substrate modification by SUMO. EMBO J. 25, 5083 – 5093.
120 Baek, S. H. (2006) A novel link between SUMO modification and cancer metastasis. Cell Cycle 5, 1492 – 1495.
121 Gutierrez, G. J. and Ronai, Z. (2006) Ubiquitin and SUMO systems in the regulation of mitotic checkpoints. Trends Biochem. Sci. 31, 324 – 332.
122 Gerondakis, S., Grumont, R., Gugasyan, R., Wong, L., Isomura, I., Ho, W. and Banerjee, A. (2006) Unravelling the complexities of the NF-kappaB signalling pathway using mouse knockout and transgenic models. Oncogene 25, 6781 – 6799.
123 Courtois, G. and Gilmore, T. D. (2006) Mutations in the NF-kappaB signaling pathway: implications for human disease. Oncogene 25, 6831 – 6843.
124 Karin, M. (2006) Nuclear factor-kappaB in cancer development and progression. Nature 441, 431 – 436.
125 Sebban, H., Yamaoka, S. and Courtois, G. (2006) Posttranslational modifications of NEMO and its partners in NF-kappaB signaling. Trends Cell Biol. 16, 569 – 577.