

# SUMO playing tag with ubiquitin

Gerrit J.K. Praefcke<sup>1</sup>, Kay Hofmann<sup>2</sup> and R. Jürgen Dohmen<sup>3</sup>

<sup>1</sup> Center for Molecular Medicine Cologne (CMCC), Institute for Genetics, University of Cologne, Cologne Biocenter, Zùlpicher StraÙe 47a, D-50674 Cologne, Germany

<sup>2</sup> Bioinformatics Group, Miltenyi Biotec GmbH, Friedrich-Ebert-StraÙe 68, D-51429 Bergisch-Gladbach, Germany

<sup>3</sup> Institute for Genetics, University of Cologne, Cologne Biocenter, Zùlpicher StraÙe 47a, D-50674 Cologne, Germany

**In addition to being structurally related, the protein modifiers ubiquitin and SUMO (small ubiquitin-related modifier), share a multitude of functional interrelations. These include the targeting of the same attachment sites in certain substrates, and SUMO-dependent ubiquitylation in others. Notably, several cellular processes, including the targeting of repair machinery to DNA damage sites, require the sequential sumoylation and ubiquitylation of distinct substrates. Some proteins promote both modifications. By contrast, the activity of some enzymes that control either sumoylation or ubiquitylation is regulated by the respective other modification. In this review, we summarize recent findings regarding intersections between SUMO and ubiquitin that influence genome stability and cell growth and which are relevant in pathogen resistance and cancer treatment.**

## Roles and intersections of the ubiquitin and SUMO protein modification systems

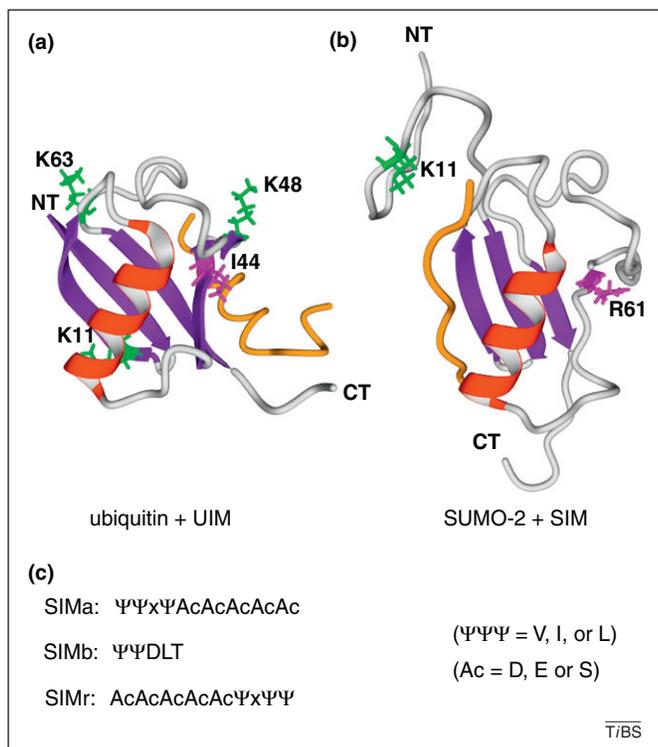
Ubiquitin is the founding member of a family of protein modifiers that are covalently attached to their substrates via isopeptide bonds formed between the carboxy group of their C-terminal glycine residues and the  $\epsilon$ -amino groups of substrate lysine residues [1]. In most cases, these modifiers affect the function of their target proteins by altering their interaction properties, either by promoting or by inhibiting binding to other proteins.

Ubiquitin is most famous for its function in targeting proteins for degradation by the 26S proteasome, which is not only important for the removal of abnormal or damaged proteins but also for many regulated processes such as the cell cycle. For a substrate to be recognized by the proteasome, ubiquitin usually needs to be attached in chains (polyubiquitylation), in which ubiquitin moieties are linked via discrete lysine residues such as K48 [2]. In addition to its role as a proteasome targeting signal, ubiquitin modification is involved in a variety of additional functions including DNA repair, signal transduction, intracellular trafficking to the lysosome/vacuole, activation of transcription factors and regulation of histones. The relevant modification in these cases is usually either a monoubiquitylation or distinct types of polyubiquitin chains such as K63-linked or linear chains [2]. A variety of structurally distinct ubiquitin binding domains have been identified, which typically bind to a hydrophobic patch of ubiquitin surrounding isoleucine 44 (I44) (Figure 1).

Similarly, SUMO modification has been implicated in many important cellular processes including the control of genome stability, signal transduction, targeting to and formation of nuclear compartments, cell cycle and meiosis [3–5]. Whereas budding yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*) have only one type of SUMO, multiple SUMO isoforms are present in many metazoans. For example, four SUMO isoforms are present in humans (Table 1). Only three of these (SUMO-1, SUMO-2 and SUMO-3) can be processed *in vivo* to bear the C-terminal diglycine motif required for post-translational conjugation. SUMO-2 and SUMO-3 are nearly identical and are assumed to be largely redundant in their functions. The analysis of SUMO substrates has revealed that the modified lysine (K) residues are often part of a (I/V/L)Kx(D/E) motif which provides a direct binding site for the SUMO conjugating enzyme UBC9 [3]. Similar to ubiquitin, SUMO can be linked to substrates in chains (polysumoylation) [6,7]. However, whereas all seven lysine residues of ubiquitin have been implicated in linkage formation [8], SUMO chains are linked mainly through a single lysine residue at position 11 in SUMO-2 and SUMO-3, which is embedded in the above-mentioned consensus sequence [6]. Similarly, the essential budding yeast SUMO has three consensus attachment sites in close proximity to each other (K11, K15 and K19) that promote chain formation *in vivo* [7]. SUMO-1, by contrast, lacks such a preferred attachment site and thus does not form chains efficiently; it might, however, serve as terminator of SUMO-2/3 chains [9]. SUMO recognition is very different from the binding of ubiquitin interacting proteins to the hydrophobic patch around I44, and is mediated by short conserved SUMO interaction motifs (SIMs), which instead bind to a surface formed by an  $\alpha$  helix and a  $\beta$  sheet on SUMO far away from the region corresponding to the hydrophobic I44 patch in ubiquitin [10,11] (Figure 1).

SUMO protein modification, which was first discovered in 1996, initially appeared to be functionally unrelated to the ubiquitin system [3]. The first indications for functional interrelations came from the observations that proteins such as I $\kappa$ B $\alpha$  (inhibitor of transcription factor NF- $\kappa$ B) and DNA polymerase processivity factor PCNA (proliferating cell nuclear antigen) were modified alternatively by SUMO or ubiquitin on specific lysine residues [12,13]. Another example is NEMO, the regulatory subunit of I $\kappa$ B kinase (IKK), which is activated by consecutive modifications with SUMO and ubiquitin upon genotoxic stress [14]. More recent discoveries summarized below have revealed

Corresponding author: Dohmen, R.J. (j.dohmen@uni-koeln.de).



**Figure 1.** Ubiquitin and SUMO share a common  $\beta$ -grasp fold but use interaction surfaces on different sides of the molecules. **(a)** Structural representation (pdb:1yx5) of human ubiquitin (colored ribbon diagram) bound to the ubiquitin interaction motif of S5a (orange tube diagram). N and C termini are indicated as NT and CT. The major chain-forming lysines K48, K63 and K11 are shown in green. I44, a crucial residue in the hydrophobic surface that mediates interaction with all known ubiquitin binding motifs is shown in magenta. **(b)** Structural representation (pdb:2awt) of human SUMO-2 (colored ribbon diagram) bound to a SUMO-interacting motif of death domain associated protein (DAXX; orange tube diagram, modeled onto SUMO-2 structure based on pdb:2kqs). The major chain-forming lysine K11 is indicated in green. K63, the residue that occupies the position corresponding to I44 in ubiquitin, but is not involved in SIM binding, is shown in magenta. **(c)** Consensus sequences of different SIM types indicating the position of hydrophobic ( $\Psi$ ) and acidic (Ac) residues. Serines were considered for the latter positions, in addition to aspartate and glutamate residues, because of their potential to be phosphorylated.

additional functional intersections between the SUMO and ubiquitin modification systems. Of particular interest in this context is the finding that sumoylation can lead to ubiquitin-dependent degradation of proteins by the proteasome [11].

### Alternative modifications by SUMO or ubiquitin direct the function of PCNA

Perhaps the best-studied example of a protein, which is directed into different functional modes by distinct SUMO or ubiquitin modifications, is PCNA. This homotrimeric protein functions as a sliding clamp on DNA where it

recruits a variety of proteins involved in replication, damage repair or recombination [15]. Monoubiquitylation of PCNA on K164 promotes error-prone DNA repair by recruiting translesion synthesis DNA polymerases [13]. Formation of a K63-linked polyubiquitin chain on the same lysine residue promotes repair of DNA damage by the ‘error-free associated with gap filling repair’ pathway, which is thought to involve a switch to the undamaged sister chromatid template. Genetic evidence suggests that not only ubiquitylation of PCNA but also its sumoylation by the Mms21 subunit (Table 1) of the structural maintenance of chromosomes (Smc)5–6 complex (see below) is required for formation of sister chromatid junctions indicative of template switching events [16]. The exact functional relationship of these PCNA modifications in this process, as well as the molecular interactions mediated by PCNA carrying K63 chains, remain unknown. Sumoylation of K164 by Siz1 (Table 1) occurring in the S phase of the cell division cycle promotes interaction with the Srs2 helicase [13,17,18]. Srs2 restricts potentially harmful unnecessary DNA recombination by preventing untimely formation of Rad51 filaments [16].

### SUMO-dependent ubiquitylation

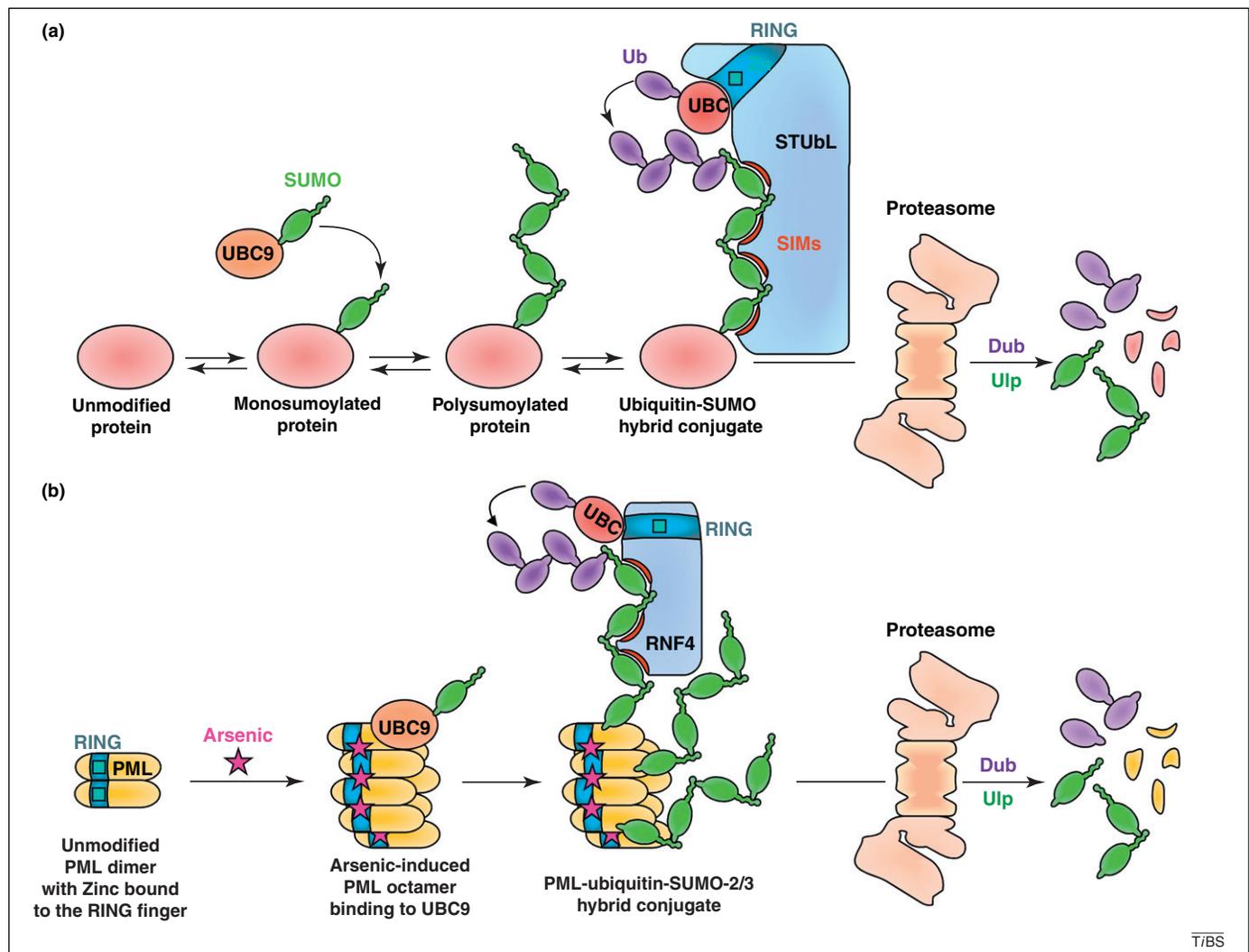
The identification of ubiquitin ligases that recognize sumoylated proteins (ULS), also known as SUMO-targeted ubiquitin ligases (STUbL), led to the discovery of a ubiquitin-dependent proteolytic control of SUMO-modified proteins (Table 1) [19–25]. The inactivation of STUbL genes in budding yeast and fission yeast, similar to inhibition of the proteasome, caused accumulation of high-molecular weight SUMO conjugates indicating that these ligases mediate a proteolytic control of SUMO conjugates [19–22]. STUbL proteins are usually characterized by the presence of multiple SIMs that mediate binding to poly-sumoylated substrates, and by their RING domains (Figure 2) which provide the ubiquitin ligase function by virtue of their interaction with ubiquitin-conjugating enzymes (UBCs).

### Yeast ubiquitin ligases for SUMO conjugates

Two STUbL systems have been identified in *S. cerevisiae*. Uls1 (also known as Ris1 because of a suspected role in silencing of mating type information) is a 184-kDa protein bearing a SWI/SNF DNA-dependent ATPase domain, which binds to Ubc4 and to sumoylated substrates, regulating their half-lives [19]. The second STUbL is a heterodimer composed of Slx5 and Slx8, two SIM-containing RING finger proteins, which are essential in the absence

**Table 1. SUMO and factors controlling its regulation**

Species	SUMO	SUMO-like domain (SLD) proteins	SUMO ligases	SUMO isopeptidases	ULS	References
<i>Saccharomyces cerevisiae</i>	Smt3	Esc2	Siz1, Siz2 (Nfi1), Mms21 (Nse2), Cst9 (Zip3)	Ulp1, Ulp2, Wss1	Uls1 (Ris1), Slx5 (Hex3)–Slx8	[1], [3–5], [5],[10], [11], [19], [22] and [64]
<i>Schizosaccharomyces pombe</i>	Pmt3	Rad60	Pli1, Nse2	Ulp1, Ulp2	Rfp1–Slx8, Rfp2–Slx8	[1], [3–5], [10], [11], [20], [21] and [30]
<i>Homo sapiens</i>	SUMO-1, -2, -3, -4	NIP45	PIAS1, -2, -3, -4, Pc2, TOPORS, RanBP2, SMCE2 (MMS21), ZIMP7 (?), ZIMP10 (?)	SENP1, -2, -3, -5, -6, -7	RNF4 (SNURF), VHL, TRIM5 $\alpha$ (?)	[1], [3–5], [10], [11], [19–21], [23–25] and [35]



**Figure 2.** Ubiquitin-dependent proteolytic control of SUMO-modified proteins. **(a)** Depicted is a simplified pathway in which polysumoylation of proteins mediated by the UBC9 SUMO-conjugating enzyme leads to recognition by a RING finger type ubiquitin ligase with SUMO interaction motifs (STUbL) and attachment of ubiquitin to the SUMO chain. Proteins modified in this manner are degraded by the proteasome. Deubiquitylation (DUB) and desumoylation (ULP) activities recycle ubiquitin and SUMO, respectively. **(b)** Proteolytic targeting of the PML (promyelocytic leukemia) protein is induced by binding to arsenic, which promotes oligomerization (often into octamers) and binding to UBC9. Polysumoylated forms of PML are recognized and ubiquitylated by the SUMO-targeted ubiquitin ligase RNF4, leading to degradation by the proteasome. Analogous targeting occurs for the oncogenic PML-RAR $\alpha$  fusion protein upon treatment with arsenic.

of the DNA helicase Sgs1 (hence the names pointing to a synthetic lethality with a defect in function x) [19,22,26]. Binding assays showed that the Slx5-Slx8 dimer selectively binds to high molecular weight SUMO conjugates, the formation of which requires SUMO polymerization via K11, K15 or K19 [19]. Genetic evidence indicates that Slx5-Slx8 is important for maintaining genomic integrity in the presence of DNA double-strand breaks (DSBs) or stalled DNA replication forks, thus explaining the synthetic lethality of *slx5* or *slx8* mutations with mutations that inactivate the DNA helicases Sgs1 or Srs2 [26]. Although the underlying substrates of Slx5-Slx8 in this context remain unknown, its function is crucial for translocating DSB sites or stalled replication forks into the vicinity of the nuclear pore complex for efficient processing [27]. These studies therefore point to additional interrelated functions of SUMO and ubiquitin in DNA maintenance beyond those that are linked to PCNA. SUMO-mediated proteolytic targeting via Slx5-Slx8 has also been shown to contribute to protein quality control [28], consistent with findings in

mammalian cells that implicated sumoylation in ubiquitin-dependent proteolytic control mechanisms under stress conditions [19,25,29].

Fission yeast has a STUbL related to budding yeast Slx5-Slx8, which has also been implicated in genome stability and DNA damage repair. This ligase is composed of Slx8, a protein homologous to *S. cerevisiae* Slx8, and one of two related RING finger proteins, Rfp1 or Rfp2 [20,21]. Similar to *S. cerevisiae* *uls1* and *slx5* mutants, *S. pombe* *slx8* mutants harbor increased amounts of SUMO conjugates. Deletion of *pli1*, which encodes a SUMO ligase, suppressed the growth defects of *S. pombe* *slx8* mutants indicating that an important function of Rfp1/2-Slx8 is to downregulate SUMO conjugates. *S. pombe* cells require Slx8 and either Rfp1 or Rfp2 for viability. In their absence, cells die owing to Chk1-dependent activation of the G2 DNA damage checkpoint. This finding indicates that Slx8, Rfp1 and Rfp2 prevent the accumulation of DNA damage. Consistent with this conclusion, *slx8* mutants often contain foci containing Rad22 (Rad52 in budding yeast), a protein

involved in homologous recombination repair (HRR). This conclusion was further supported by genetic data showing synthetic lethality between *slx8* and mutations in HRR genes [20]. A putative substrate of Rfp1/2–Slx8 is the conserved Rad60 protein (Table 1), which is involved in HRR together with the dual specificity ubiquitin/SUMO ligase Smc5–Smc6 complex (see below). Rad60 binds Rfp1 via its SUMO-like domains (SLDs) and is ubiquitinated by Rfp1–Slx8 *in vitro* [20]; however, Rfp1/2–Slx8-dependent ubiquitination of Rad60 has not been detected *in vivo* [30]. It is also possible that Rad60 negatively regulates Rfp1/2–Slx8 by competing with SUMO binding, in a manner similar to its interaction with Ubc9. The ability of Ubc9 to promote chain formation requires noncovalent binding of SUMO to an internal binding site. Masking of this site by binding to Rad60 inhibits chain formation and is thought to instead promote monosumoylation in a Mms21-dependent pathway [31,32]. Together, these findings indicate that Rad60 contributes to directing substrates into STUbL-independent pathways.

#### RNF4

The dramatic accumulation of SUMO-2/3 conjugates upon inhibition of the proteasome in human cells indicated that STUbL-mediated proteolytic control of polymeric SUMO conjugates is conserved from yeasts to mammals [19]. The nuclear RING finger protein RNF4, which binds to SUMO and localizes to PML (promyelocytic leukemia) nuclear bodies (Box 1), displays sequence homology to Rfp1 and Rfp2 and complements deletions of STUbL genes in yeast [19–21]. Polysumoylated PML was identified as the first physiological substrate of RNF4 [23–25]. Ubiquitylation of PML or the oncogenic PML–retinoic acid receptor (RAR)  $\alpha$  fusion protein is stimulated by arsenic and occurs preferentially on the SUMO chain rather than on PML itself (Figure 2 and Box 1). RNF4 functions as a homodimer that recognizes polymeric SUMO chains by multiple SIMs in its N-terminal half [23,33,34]. In addition to PML, the kinetochore protein CENP-I and the hypoxia inducible factor 2 $\alpha$  (HIF2 $\alpha$ ) have recently been identified as additional SUMO-dependent substrates targeted for degradation by RNF4 [35,36]. A proteomic analysis of polysumoylated proteins interacting with the SIM domain of RNF4 identified several hundred additional putative substrates [37]. Many of them, including DNA repair proteins, were induced by heat stress, which stimulates the formation of high-molecular weight SUMO-2/3 conjugates [38]. Collectively, these findings suggest that STUbL-mediated degradation of polysumoylated proteins participates in diverse cellular stress responses. Another recent study identified RNF4 as a factor essential for embryogenesis that promotes DNA demethylation [39]. The latter function of RNF4 is depending on its SIM and RING domains suggesting that its STUbL activity is involved. This study also showed, however, that RNF4 interacts with thymidine DNA glycosylase (TDG) and apurinic/aprimidinic (AP) site endonuclease (APE) in a SIM-independent manner. Both TDG and APE were shown to be required for RNF4-stimulated DNA demethylation. These data suggested that RNF4, in addition to its STUbL activity, also has SUMO-independent activities. Similar observations were

#### Box 1. PML and nuclear bodies

Protein PML (also known as TRIM19), which belongs to the tripartite motif (TRIM) family, was identified in cells of patients suffering from acute promyelocytic leukemia (APL) which express a fusion protein between the PML protein and RAR $\alpha$  [72]. Normal cells express up to 11 isoforms of PML that localize in distinctive puncta in the nucleus, which are called PML nuclear bodies (PML-NBs [73]).

The formation of PML-NBs requires PML sumoylation and oligomerization via the TRIM domain. Sumoylated PML serves as a docking station for many proteins, most of which are themselves sumoylated and/or contain SIMs. Furthermore, PML was reported to bind UBC9 via its RING finger and to promote sumoylation [72,74]. PML-NBs are very dynamic structures that rapidly exchange components (including PML isoforms) with the surrounding nucleoplasm. An exception is the PML2 isoform, which serves as a scaffolding protein [75]. PML associates with DNA, including certain chromatin regions, DNA breaks, telomeres and viral DNA [72,76]. A recent study showed that sumoylated PML and Sp100 form the outer shell of PML-NBs which either contain DNA or SUMO-2 in their core [77].

PML-NBs have been implicated in diverse functions including transcription, DNA repair, DNA replication and RNA transport [72], but they have also been ascribed a purely passive role as intranuclear depots [78]. Furthermore, PML-NBs have an antiviral function and PML, Sp100 and other PML-NB localized proteins are upregulated by interferons. Several viruses, however, encode proteins that impair the integrity of PML-NBs by disassembly or degradation of constituent proteins [76]. Interesting examples of such proteins are the related varicella zoster virus ORF61 and herpes simplex ICP0, both ubiquitin ligases and likely STUbL candidates [79]. The targets of ICP0 includes PML, Sp100 as well as RNF8 and RNF168 [80], which are involved in the assembly of DNA repair sites (Figure 3).

The proteasomal degradation of PML and the oncogenic PML–RAR $\alpha$  fusion protein can be enhanced by arsenic, which is used for the treatment of APL patients [81]. Importantly, this degradation is preceded by an increased SUMO-2/3 modification that targets these proteins for RNF4-mediated ubiquitylation (Figure 2) [23–25,82]. Arsenic and other stress factors enhance phosphorylation of PML [72,83], thereby triggering PML oligomerization by oxidation and substitution of the zinc ions in the TRIM [84,85]. This in turn promotes binding of SUMO-loaded UBC9 leading to polysumoylation of PML and subsequent SUMO-dependent ubiquitylation by RNF4.

reported recently for the RNF4-related *Drosophila* protein degradingolade (Dgrn), a STUbL with a role in transcriptional control during embryonic development, which also appears to have SUMO-dependent and SUMO-independent functions [40,41]. These findings are furthermore consistent with data on the *S. cerevisiae* STUbL Slx5–Slx8, which was shown to promote degradation of the transcription factor MAT $\alpha$ 2 in a SUMO-independent manner [42]. In this case, however, the SIMs were involved in direct recognition of the substrate. These examples illustrate that the characterization of a ubiquitin ligase as a STUbL does not necessarily cover all its functions.

#### Regulation of hypoxia inducible factors (HIFs) by sumoylation and the VHL ubiquitin ligase

HIFs regulate the response of mammalian cells to changing oxygen conditions [43]. The active forms of these transcription factors are heterodimers composed of HIF $\alpha$  (HIF1 $\alpha$ , HIF2 $\alpha$  or HIF3 $\alpha$ ) and HIF $\beta$  (HIF1 $\beta$ , also called ARNT). Under normal conditions (normoxia), HIF $\alpha$  is hydroxylated by prolyl hydroxylases leading to its ubiquitylation in the cytosol by an E3 ubiquitin ligase complex containing the von Hippel–Lindau (VHL) protein and degradation by

the proteasome. Under low oxygen conditions (hypoxia), HIF $\alpha$  is not hydroxylated and is therefore able to accumulate in the nucleus where it dimerizes with constitutively expressed HIF $\beta$  and activates its target genes. It is clear that HIF $\alpha$  is sumoylated under hypoxia, but the functional consequences of this modification remain controversial.

Inactivation of the SUMO protease SENP1 has been reported to promote HIF1 $\alpha$  degradation during hypoxia, suggesting that sumoylation triggers HIF1 $\alpha$  degradation [44]. Surprisingly, proteolytic targeting of HIF1 $\alpha$  in hypoxic conditions appears to depend on its modification by SUMO-1 as well as on VHL-mediated ubiquitylation, thus indicating that VHL might be a STUbL specific for SUMO-1. In addition, several studies suggested that SUMO-1 modification inhibits HIF1 $\alpha$  activity [45,46]. A similar scenario has been described for HIF2 $\alpha$ , but proteolytic targeting of HIF2 $\alpha$  during hypoxia involves modification with SUMO-2/3, which precedes its ubiquitylation by either VHL or RNF4 [36].

In contrast to these findings, RSUME (RWD-sumoylation enhancer) has been described to stabilize HIF1 $\alpha$  and to increase its transcriptional activity [47]. RSUME is itself induced by hypoxia and enhances the activity of UBC9, thereby resulting in the increased sumoylation of several targets, including HIF1 $\alpha$ .

These seemingly discrepant findings might reflect the multitude and complexity of HIF1 regulating mechanisms, including nuclear translocation, sumoylation and desumoylation, as well as SUMO-dependent or -independent ubiquitylation. In line with this notion, VHL itself is regulated both by sumoylation and ubiquitylation in a complex manner. Protein inhibitor of activated STAT 4 (PIAS4)-mediated sumoylation, which is upregulated during hypoxia, induces VHL dimerization thereby inhibiting its assembly into a functional ubiquitin ligase complex. Interestingly, however, PIAS4-mediated sumoylation also stabilizes VHL by blocking its ubiquitylation (on the same lysine residues) and its subsequent nuclear export [48]. VHL is thus an example of a ubiquitin pathway enzyme which is antagonistically modified with either SUMO or ubiquitin.

### Interplay of SUMO and ubiquitin modification in DNA double-strand break repair in mammals

The response to DNA DSBs, in which a variety of proteins are recruited to the damage site resulting in the formation of DNA repair foci, provides an intriguing example of a process in which SUMO and ubiquitin modifications contribute to the coordination of the action of multiple factors (Figure 3) [49]. As an early step in this process, the activation of DNA damage induced protein kinases ATM (ataxia telangiectasia mutated), ATR (ataxia telangiectasia and Rad3 related) and DNA-dependent protein kinase (DNA-PK) trigger phosphorylation of histone H2AX. Phosphorylated H2AX, a marker of DNA repair foci also known as  $\gamma$ -H2AX, recruits MDC1 (mediator of DNA damage checkpoint 1), which itself promotes the accumulation of the ubiquitin ligase RNF8. This ligase cooperates with UBC13 to promote ubiquitylation of histones H2A and H2AX (and probably other proteins). Ubiquitylated histones in turn attract the ubiquitin ligase RNF168, which modifies RAP80, a factor involved in the recruitment of the

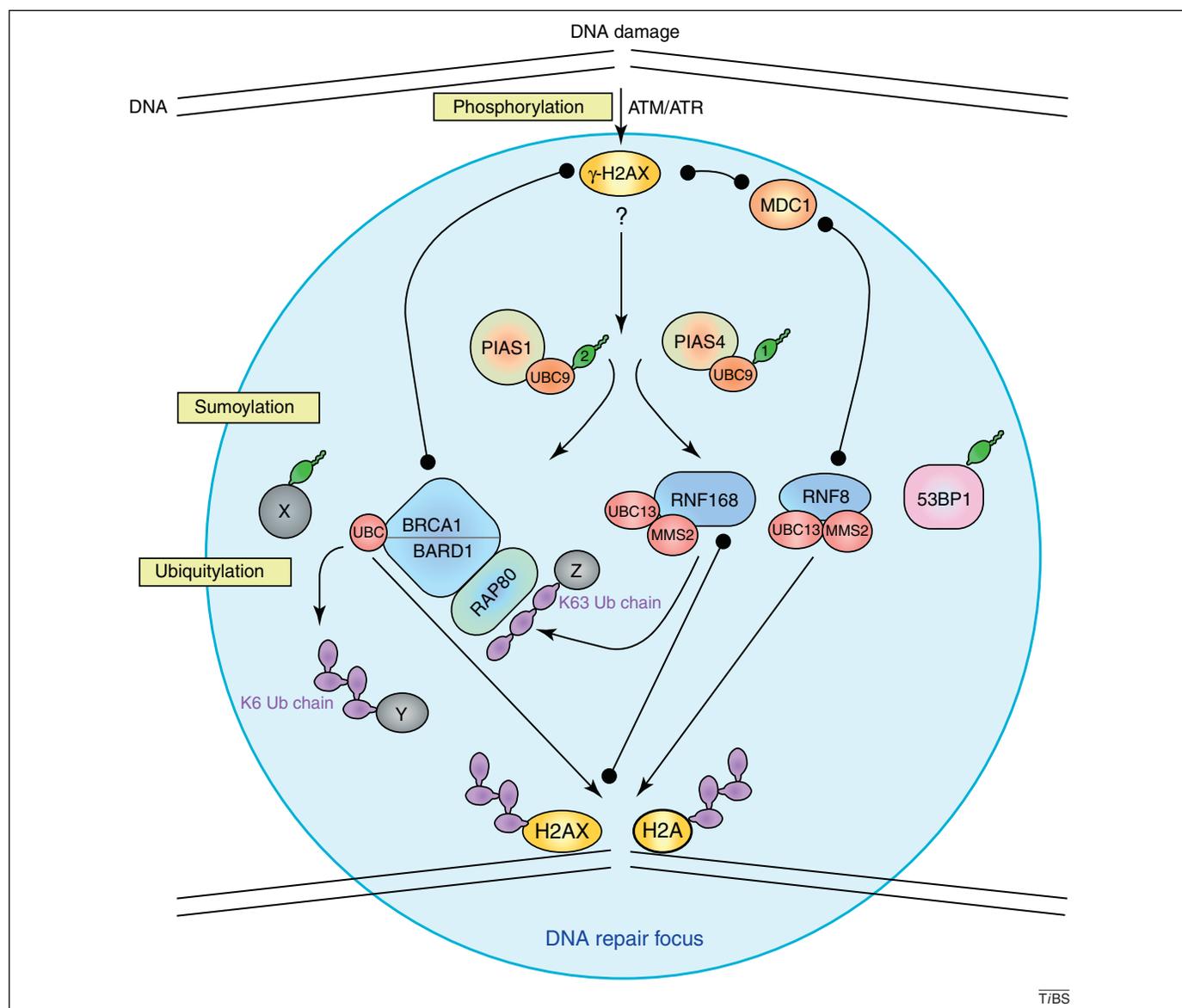
breast cancer 1, early onset (BRCA1) ubiquitin ligase complex. BRCA1 is an important factor in the marking of DNA damage sites by modification of histone H2AX and other still unidentified proteins. Recent findings demonstrate that the SUMO ligases PIAS1 and PIAS4, as well as the SUMO-conjugating enzyme UBC9, are also recruited to DNA repair foci at a relatively early step in the process [50,51]. These enzymes mediate either SUMO-1 or SUMO-2/3 modification of proteins such as p53BP1 (p53 binding protein 1) and the ubiquitin ligases BRCA1 as well as of additional still unidentified proteins. Sumoylation of these proteins promotes their recruitment to and/or retention at DNA repair foci, which is important for DSB repair. Indeed, the ability of BRCA1 to modify its substrates with K6-linked ubiquitin chains relies upon its sumoylation characterizing it as a SUMO-regulated ubiquitin ligase [50].

These findings provide important insights into an as of yet still incomplete picture of a complex interaction network involving SUMO and ubiquitin modifications in establishing repair sites in response to DSBs, to which enzymes that execute DNA damage repair and recombination are recruited (Figure 3). It will be interesting to study whether STUbL activity is also involved in establishing or controlling DNA repair sites. An important question in this context is how the disassembly of repair sites is coordinated after the completion of damage repair. Probable mechanisms include deubiquitylation and desumoylation, as well as SUMO-dependent proteolytic targeting. The STUbL RNF4 is a candidate factor for mediating the latter process. Putative substrates include the SUMO-modified ubiquitin ligases that coordinate formation of repair sites (Figure 3). A role of STUbL activity in the regulation of DNA repair sites is supported by the observation that Slx5 localizes to DNA repair foci in *S. cerevisiae* [52].

### Enzymes acting on SUMO and ubiquitin

UBC9 is as closely related to the ubiquitin E2 conjugating enzymes as they are to each other; thus, a certain degree of crosstalk at the conjugation level could have been expected. Yet few, if any, clear examples for ubiquitin ligases collaborating with UBC9 or SUMO ligases collaborating with a different UBC are known. There are some established cases where one protein or protein complex can transfer both ubiquitin and SUMO onto a target. However, all of the known examples employ different protein regions (or even different complex subunits) for dealing with the distinct UbL specific conjugating enzymes.

One important example is the topoisomerase-1 interacting protein TOPORS, which contains a RING finger that can confer ubiquitin ligase activity by recruiting the E2 enzymes UBCH5 or UBCH6 [53]. By contrast, its sumoylation activity is independent of the N-terminal RING finger but requires a central region that comprises a SIM [54]. Notably, TOPORS is able to both ubiquitylate and sumoylate the tumor suppressor p53 [53,54]. p53 is stabilized by TOPORS-mediated sumoylation, whereas TOPORS-mediated ubiquitylation has a destabilizing effect. The two modification activities appear to be differentially regulated by phosphorylation. The polo-like kinase PLK1 phosphorylates TOPORS on Ser-718, leading to decreased sumoylation and



**Figure 3.** Interrelation of SUMO and ubiquitin modifications in the mammalian response to DNA double-strand breaks (DSBs). DSBs lead to the formation of DNA repair foci (indicated by the large blue circle in the background) in response to the activation of damage response protein kinases, which phosphorylate a variety of proteins including histones H2AX (the phosphorylated form is called  $\gamma$ -H2AX). MDC1 (mediator of DNA damage checkpoint protein 1) and BRCA1 bind  $\gamma$ -H2AX (indicated by lines ending in filled circles). MDC1 in turn recruits the ubiquitin ligase RNF8, leading to ubiquitylation of histones, which results in binding of RNF168, another ubiquitin ligase. RNF168 modifies substrates by attaching K63-linked ubiquitin chains, which bind RAP80, a BRCA1 recruitment factor. The BRCA1 complex generates K6-linked ubiquitin chains. By an as yet unidentified mechanism, ATM/ATR signaling also promotes the recruitment of the sumoylating enzymes PIAS1, PIAS4 and UBC9 to DNA repair foci. Among the proteins that are either modified by SUMO-1 or SUMO-2/3 are the BRCA1-BARD1 complex and 53BP1, as well as other uncharacterized proteins. Sumoylation promotes recruitment and/or maintenance of these proteins at the repair foci and thereby induces the ubiquitylation of various proteins including the histones H2A and H2AX.

increased ubiquitylation activity [55]. The same modification also triggers TOPORS degradation [56]. Moreover, phosphorylation at Ser-98 by an unknown kinase stimulates the ubiquitylation activity of TOPORS without affecting its ability to sumoylate target proteins [57], which include DNA topoisomerase-1 [58], chromatin regulators such as SIN3A [59] and IKK- $\epsilon$  (Box 1) [60,61]. TOPORS can also undergo autosumoylation, which appears to result in its stabilization, possibly by antagonizing ubiquitylation [62].

The conserved 'structural maintenance of chromosomes' (Smc5-6) complex involved in sister chromatid cohesion, mitotic chromosome condensation and DNA repair is an example of a multiprotein complex that harbors both ubiquitylation and sumoylation activities. The ubiquitin ligase activity has been assigned to a subcomplex containing

the RING finger protein Nse1 [63], whereas sumoylation activity is mediated by Mms21 [64]. Mms21-mediated sumoylation of Smc5-6 complex subunits is thought to control its ligase functions and its localization to distinct chromosomal areas [64]. Interestingly, the Smc5-6 complex interacts with SLD protein Rad60 but also, via its Nse5 subunit, with Rfp1-Slx8 (Slx5-Slx8 in budding yeast) [10] indicating that this complex is a meeting point of multiple SUMO-ubiquitin interactions.

A recent report characterized the SIM-containing budding yeast Wss1 protein as a metalloprotease that *in vitro* can remove ubiquitin moieties attached to SUMO chains as well as depolymerize SUMO chains. Wss1, which was shown to be associated with the proteasome, is required for ubiquitin-dependent proteolytic control of polysumoylated

substrates [65]. It remains unclear, however, which of the two specificities assigned to Wss1, if relevant *in vivo*, are important for this function in the proteolysis of SUMO-ubiquitin hybrid conjugates. Another possibility is that Wss1, in addition to its protease activity, provides an additional receptor site at the proteasome by virtue of its specific recognition of substrates characterized by the presence of ubiquitin-SUMO linkages.

### SUMO-dependent regulation of enzymes of the ubiquitin system and vice versa

A direct way of generating crosstalk between SUMO and ubiquitin-based signaling is the regulatory conjugation of one modifier to enzymes of the other modification system. A large number of cytoplasmic and nuclear signaling proteins become ubiquitylation targets at one point of their life cycle, and the enzymes of the sumoylation/desumoylation cascade should be no exception. Surprisingly, however, two SUMO-specific isopeptidases are the only examples for which such a regulatory pattern has been studied in detail. SENP3 is an isopeptidase responsible for the desumoylation of nuclear proteins such as the HIF1 coactivator p300 [66]. Whereas SENP3 is continuously degraded by the ubiquitin-proteasome system under normal conditions, reactive oxygen species (ROS) inhibit this degradation, leading to HIF1 activation via p300 desumoylation under mild oxidative stress [66]. This mechanism contributes to the complexity of HIF1 regulation by SUMO and ubiquitin. The related protease SENP2, which shuttles between the nucleus and the cytosol, is targeted for ubiquitin-dependent degradation in the latter compartment [67].

Conversely, there are several reported examples of ubiquitin cascade components that are regulated by sumoylation. The ubiquitin-conjugating enzyme E2-25k is a C-terminally elongated E2 with a ubiquitin-binding UBA domain that supports the formation of unanchored K48-linked polyubiquitin chains. Unexpectedly, E2-25k was found to be modified by SUMO-1 on K14 within the core domain, which inhibits the formation of free ubiquitin chains [68]. More recently, UBC9 was reported to undergo autosumoylation at a position analogous to the sumoylation site of E2-25k [69]; this modification alters UBC9 target specificity. It will be interesting to determine if sumoylated E2-25k also displays a shift towards SIM-containing targets or E3 ligases.

Finally, USP25 is a ubiquitin-specific protease that is regulated by sumoylation. The region N-terminal (or upstream) of the catalytic domain contains three predicted ubiquitin-binding domains: one UBA domain followed by two ubiquitin-interacting motifs (UIMs). The first UIM overlaps with a predicted SIMb motif, which recognizes SUMO-2/3 and promotes USP25 sumoylation at K99 within the UIM [70]. More recent results indicate that the same K99 residue can also be ubiquitylated, a process that is aided and limited to the monoubiquitin stage by the ensemble of ubiquitin binding domains [71]. At least in the case of the model substrate MyBPC1 (myosin-binding protein C), K99 ubiquitylation enhances USP25 activity, whereas sumoylation at the same position has the opposite effect [72]. It is interesting to note that USP28 has the same domain architecture as USP25, including the predicted

SIMb overlapping with a UIM. There are no data regarding USP28 sumoylation, but it is possible that USP25 is not the only ubiquitin-specific protease regulated by alternative SUMO or ubiquitin modifications.

### Concluding remarks and future perspectives

The examples described in this article provide only a glimpse of the complex and intertwined relations between SUMO and ubiquitin, many of which are involved in the response to DNA damage and other stresses. This complexity often makes it difficult to study the effects of a particular modification in a cellular context, given that interventions that affect either of these modifications can have complicated indirect effects on the proteins or processes under investigation. In the future, detailed dissections of the regulation and functional impact of these modifications on individual proteins as well as proteomic analyses will be required to obtain a deeper understanding of the astonishingly complex network of SUMO-ubiquitin interactions.

In addition to the identification of substrates that are controlled by SUMO and ubiquitin, a key question relates to the exact topologies of hybrid ubiquitin-SUMO modifications on individual substrates. In cases in which such modifications result in proteolytic targeting, it will be important to understand how the proteasome recognizes and handles these complex substrates ultimately leading to their degradation and recycling of the modifiers. Another challenge for the future will be to identify and characterize novel STUbLs and their activities.

### Acknowledgments

We thank three anonymous reviewers for their helpful critique on the manuscript. This work is supported by grants from the Deutsche Forschungsgemeinschaft within the priority program SPP1365 and the Köln Fortune program.

### References

- 1 Kerscher, O. *et al.* (2006) Modification of proteins by ubiquitin and ubiquitin-like proteins. *Annu. Rev. Cell. Dev. Biol.* 22, 159–180
- 2 Komander, D. (2009) The emerging complexity of protein ubiquitination. *Biochem. Soc. Trans.* 37, 937–953
- 3 Johnson, E.S. (2004) Protein modification by SUMO. *Annu. Rev. Biochem.* 73, 355–382
- 4 Geiss-Friedlander, R. and Melchior, F. (2007) Concepts in sumoylation: a decade on. *Nat. Rev. Mol. Cell Biol.* 8, 947–956
- 5 Ulrich, H.D. (2009) The SUMO system: an overview. *Methods Mol. Biol.* 497, 3–16
- 6 Tatham, M.H. *et al.* (2001) Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9. *J. Biol. Chem.* 276, 35368–35374
- 7 Bylebyl, G.R. *et al.* (2003) The SUMO isopeptidase Ulp2 prevents accumulation of SUMO chains in yeast. *J. Biol. Chem.* 278, 44112–44120
- 8 Xu, P. *et al.* (2009) Quantitative proteomics reveals the function of unconventional ubiquitin chains in proteasomal degradation. *Cell* 137, 133–145
- 9 Matic, I. *et al.* (2007) In vivo identification of human SUMO polymerization sites by high accuracy mass spectrometry and an in vitro to in vivo strategy. *Mol. Cell. Proteomics* 7, 132–144
- 10 Perry, J.J. *et al.* (2008) A SIM-ultaneous role for SUMO and ubiquitin. *Trends Biochem. Sci.* 33, 201–208
- 11 Miteva, M. *et al.* (2010) Sumoylation as a signal for polyubiquitylation and proteasomal degradation. *Subcell. Biochem.* 54, 195–214
- 12 Desterro, J.M. *et al.* (1998) SUMO-1 modification of I $\kappa$ B $\alpha$  inhibits NF- $\kappa$ B activation. *Mol. Cell* 2, 233–239
- 13 Hoege, C. *et al.* (2002) RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. *Nature* 419, 135–141

## Review

- 14 Huang, T.T. *et al.* (2003) Sequential modification of NEMO/IKK $\gamma$  by SUMO-1 and ubiquitin mediates NF- $\kappa$ B activation by genotoxic stress. *Cell* 115, 565–576
- 15 Moldovan, G.L. *et al.* (2007) PCNA, the maestro of the replication fork. *Cell* 129, 665–679
- 16 Branzei, D. *et al.* (2008) SUMOylation regulates Rad18-mediated template switch. *Nature* 456, 915–920
- 17 Pfander, B. *et al.* (2005) SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. *Nature* 436, 428–433
- 18 Papouli, E. *et al.* (2005) Crosstalk between SUMO and ubiquitin on PCNA is mediated by recruitment of the helicase Srs2p. *Mol. Cell* 19, 123–133
- 19 Uzunova, K. *et al.* (2007) Ubiquitin-dependent proteolytic control of SUMO conjugates. *J. Biol. Chem.* 282, 34167–34175
- 20 Prudden, J. *et al.* (2007) SUMO-targeted ubiquitin ligases in genome stability. *EMBO J.* 26, 4089–4101
- 21 Sun, H. *et al.* (2007) Conserved function of RNF4 family proteins in eukaryotes: targeting a ubiquitin ligase to SUMOylated proteins. *EMBO J.* 26, 4102–4112
- 22 Xie, Y. *et al.* (2007) The yeast HEX3-SLX8 heterodimer is a ubiquitin ligase stimulated by substrate sumoylation. *J. Biol. Chem.* 282, 34176–34184
- 23 Tatham, M.H. *et al.* (2008) RNF4 is a poly-SUMO-specific E3 ubiquitin ligase required for arsenic-induced PML degradation. *Nat. Cell Biol.* 10, 538–546
- 24 Lallemand-Breitenbach, V. *et al.* (2008) Arsenic degrades PML or PML-RAR $\alpha$  through a SUMO-triggered RNF4/ubiquitin-mediated pathway. *Nat. Cell Biol.* 10, 547–555
- 25 Weisshaar, S.R. *et al.* (2008) Arsenic trioxide stimulates SUMO-2/3 modification leading to RNF4-dependent proteolytic targeting of PML. *FEBS Lett.* 582, 3174–3178
- 26 Mullen, J.R. *et al.* (2001) Requirement for three novel protein complexes in the absence of the Sgs1 DNA helicase in *Saccharomyces cerevisiae*. *Genetics* 157, 103–118
- 27 Nagai, S. *et al.* (2008) Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. *Science* 322, 597–602
- 28 Wang, Z. and Prelich, G. (2009) Quality control of a transcriptional regulator by SUMO-targeted degradation. *Mol. Cell Biol.* 29, 1694–1706
- 29 Tatham, M.H. *et al.* (2011) Comparative proteomic analysis identifies a role for SUMO in protein quality control. *Sci. Signal.* 4, rs4
- 30 Heideker, J. *et al.* (2009) Genome stability roles of SUMO-targeted ubiquitin ligases. *DNA Repair* 8, 517–524
- 31 Sekiyama, N. *et al.* (2010) Structural basis for regulation of poly-SUMO chain by a SUMO-like domain of Nip45. *Proteins* 78, 1491–1502
- 32 Heideker, J. *et al.* (2011) SUMO-targeted ubiquitin ligase, Rad60, and Nse2 SUMO ligase suppress spontaneous Top1-mediated DNA damage and genome instability. *PLoS Genet.* 7, e1001320
- 33 Liew, C.W. *et al.* (2010) RING domain dimerization is essential for RNF4 function. *Biochem. J.* 431, 23–29
- 34 Plechanovova, A. *et al.* (2011) Mechanism of ubiquitylation by dimeric RING ligase RNF4. *Nat. Struct. Mol. Biol.* 18, 1052–1059
- 35 Mukhopadhyay, D. *et al.* (2010) The SUMO protease SENP6 is essential for inner kinetochore assembly. *J. Cell Biol.* 188, 681–692
- 36 van Hagen, M. *et al.* (2010) RNF4 and VHL regulate the proteasomal degradation of SUMO-conjugated hypoxia-inducible factor-2 $\alpha$ . *Nucleic Acids Res.* 38, 1922–1931
- 37 Bruderer, R. *et al.* (2011) Purification and identification of endogenous polySUMO conjugates. *EMBO Rep.* 12, 142–148
- 38 Golebiowski, F. *et al.* (2009) System-wide changes to SUMO modifications in response to heat shock. *Sci. Signal.* 2, ra24
- 39 Hu, X.V. *et al.* (2010) Identification of RING finger protein 4 (RNF4) as a modulator of DNA demethylation through a functional genomics screen. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15087–15092
- 40 Abed, M. *et al.* (2011) Degringolade, a SUMO-targeted ubiquitin ligase, inhibits Hairy/Groucho-mediated repression. *EMBO J.* 30, 1289–1301
- 41 Barry, K.C. *et al.* (2011) The Drosophila STUbL protein Degringolade limits HES functions during embryogenesis. *Development* 138, 1759–1769
- 42 Xie, Y. *et al.* (2010) SUMO-independent in vivo activity of a SUMO-targeted ubiquitin ligase toward a short-lived transcription factor. *Genes Dev.* 24, 893–903
- 43 Lee, F.S. and Percy, M.J. (2011) The HIF pathway and erythrocytosis. *Annu. Rev. Pathol.* 6, 165–192
- 44 Cheng, J. *et al.* (2007) SUMO-specific protease 1 is essential for stabilization of HIF1 $\alpha$  during hypoxia. *Cell* 131, 584–595
- 45 Berta, M.A. *et al.* (2007) SUMOylation of hypoxia-inducible factor-1 $\alpha$  reduces its transcriptional activity. *Biochem. Biophys. Res. Commun.* 360, 646–652
- 46 Kang, X. *et al.* (2010) PIASy stimulates HIF1 $\alpha$  SUMOylation and negatively regulates HIF1 $\alpha$  activity in response to hypoxia. *Oncogene* 29, 5568–5578
- 47 Carbia-Nagashima, A. *et al.* (2007) RSUME, a small RWD-containing protein, enhances SUMO conjugation and stabilizes HIF-1 $\alpha$  during hypoxia. *Cell* 131, 309–323
- 48 Cai, Q. and Robertson, E.S. (2010) Ubiquitin/SUMO modification regulates VHL protein stability and nucleocytoplasmic localization. *PLoS ONE* 5, e12636
- 49 Ulrich, H.D. and Walden, H. (2010) Ubiquitin signalling in DNA replication and repair. *Nat. Rev. Mol. Cell Biol.* 11, 479–489
- 50 Galanty, Y. *et al.* (2009) Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. *Nature* 462, 935–939
- 51 Morris, J.R. *et al.* (2009) The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. *Nature* 462, 886–890
- 52 Cook, C.E. *et al.* (2009) The SUMO-targeted ubiquitin ligase subunit Slx5 resides in nuclear foci and at sites of DNA breaks. *Cell Cycle* 8, 1080–1089
- 53 Rajendra, R. *et al.* (2004) Topors functions as an E3 ubiquitin ligase with specific E2 enzymes and ubiquitinates p53. *J. Biol. Chem.* 279, 36440–36444
- 54 Weger, S. *et al.* (2005) Topors acts as a SUMO-1 E3 ligase for p53 in vitro and in vivo. *FEBS Lett.* 579, 5007–5012
- 55 Yang, X. *et al.* (2009) Plk1-mediated phosphorylation of Topors regulates p53 stability. *J. Biol. Chem.* 284, 18588–18592
- 56 Yang, X. *et al.* (2010) Plk1 phosphorylation of Topors is involved in its degradation. *Mol. Biol. Rep.* 37, 3023–3028
- 57 Park, H.-J. *et al.* (2008) Identification of phosphorylation sites of TOPORS and a role for serine 98 in the regulation of ubiquitin but not SUMO E3 ligase activity. *Biochemistry* 47, 13887–13896
- 58 Hammer, E. *et al.* (2007) The E3 ligase Topors induces the accumulation of polysumoylated forms of DNA topoisomerase I in vitro and in vivo. *FEBS Lett.* 581, 5418–5424
- 59 Pungaliya, P. *et al.* (2007) TOPORS functions as a SUMO-1 E3 ligase for chromatin-modifying proteins. *J. Proteome Res.* 6, 3918–3923
- 60 Rasheed, Z.A. *et al.* (2002) The topoisomerase I-binding RING protein, topors, is associated with promyelocytic leukemia nuclear bodies. *Exp. Cell Res.* 277, 152–160
- 61 Renner, F. *et al.* (2010) SUMOylation-dependent localization of IKK $\epsilon$  in PML nuclear bodies is essential for protection against DNA-damage-triggered cell death. *Mol. Cell* 37, 503–515
- 62 Weger, S. *et al.* (2003) The DNA topoisomerase I binding protein topors as a novel cellular target for SUMO-1 modification: characterization of domains necessary for subcellular localization and sumolation. *Exp. Cell Res.* 290, 13–27
- 63 Doyle, J.M. *et al.* (2010) MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases. *Mol. Cell* 39, 963–974
- 64 Stephan, A.K. *et al.* (2011) The Nse2/Mms21 SUMO ligase of the Smc5/6 complex in the maintenance of genome stability. *FEBS Lett.* 585, 2907–2913
- 65 Mullen, J.R. *et al.* (2010) Wss1 is a SUMO-dependent isopeptidase that interacts genetically with the Slx5–Slx8 SUMO-targeted ubiquitin ligase. *Mol. Cell Biol.* 30, 3737–3748
- 66 Huang, C. *et al.* (2009) SENP3 is responsible for HIF-1 transactivation under mild oxidative stress via p300 de-SUMOylation. *EMBO J.* 28, 2748–2762
- 67 Itahana, Y. *et al.* (2006) Nucleocytoplasmic shuttling modulates activity and ubiquitination-dependent turnover of SUMO-specific protease 2. *Mol. Cell Biol.* 26, 4675–4689

## Review

- 68 Pichler, A. *et al.* (2005) SUMO modification of the ubiquitin-conjugating enzyme E2-25K. *Nat. Struct. Mol. Biol.* 12, 264–269
- 69 Knipscheer, P. *et al.* (2008) Ubc9 sumoylation regulates SUMO target discrimination. *Mol. Cell* 31, 371–382
- 70 Meulmeester, E. *et al.* (2008) Mechanism and consequences for paralog-specific sumoylation of ubiquitin-specific protease 25. *Mol. Cell* 30, 610–619
- 71 Denuc, A. *et al.* (2009) The UBA-UIM domains of the USP25 regulate the enzyme ubiquitination state and modulate substrate recognition. *PLoS ONE* 4, e5571
- 72 Bernardi, R. and Pandolfi, P.P. (2007) Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies. *Nat. Rev. Mol. Cell Biol.* 8, 1006–1016
- 73 Nisole, S. *et al.* (2005) TRIM family proteins: retroviral restriction and antiviral defence. *Nat. Rev. Microbiol.* 3, 799–808
- 74 Chu, Y. and Yang, X. (2011) SUMO E3 ligase activity of TRIM proteins. *Oncogene* 30, 1108–1116
- 75 Weidtkamp-Peters, S. *et al.* (2008) Dynamics of component exchange at PML nuclear bodies. *J. Cell. Sci.* 121, 2731–2743
- 76 Tavalai, N. and Stamminger, T. (2008) New insights into the role of the subnuclear structure ND10 for viral infection. *Biochim. Biophys. Acta* 1783, 2207–2221
- 77 Lang, M. *et al.* (2010) Three-dimensional organization of promyelocytic leukemia nuclear bodies. *J. Cell Sci.* 123, 392–400
- 78 Negorev, D. and Maul, G.G. (2001) Cellular proteins localized at and interacting within ND10/PML nuclear bodies/PODs suggest functions of a nuclear depot. *Oncogene* 20, 7234–7242
- 79 Wang, L. *et al.* (2011) Disruption of PML nuclear bodies is mediated by ORF61 SUMO-interacting motifs and required for varicella-zoster virus pathogenesis in skin. *PLoS Pathog.* 7, e1002157
- 80 Lilley, C.E. *et al.* (2011) The intrinsic antiviral defense to incoming HSV-1 genomes includes specific DNA repair proteins and is counteracted by the viral protein ICP0. *PLoS Pathog.* 7, e1002084
- 81 Wang, Z.Y. and Chen, Z. (2008) Acute promyelocytic leukemia: from highly fatal to highly curable. *Blood* 111, 2505–2515
- 82 Geoffroy, M.C. *et al.* (2010) Arsenic-induced, SUMO-dependent recruitment of RNF4 into PML nuclear bodies. *Mol. Biol. Cell* 21, 4227–4239
- 83 Stehmeier, P. and Muller, S. (2009) Phospho-regulated SUMO interaction modules connect the SUMO system to CK2 signaling. *Mol. Cell* 33, 400–409
- 84 Zhang, X.W. *et al.* (2010) Arsenic trioxide controls the fate of the PML-RAR $\alpha$  oncoprotein by directly binding PML. *Science* 328, 240–243
- 85 Jeanne, M. *et al.* (2010) PML/RARA oxidation and arsenic binding initiate the antileukemia response of As<sub>2</sub>O<sub>3</sub>. *Cancer Cell* 18, 88–98