Review **Open Access**

Andrea Pichler*, Chronis Fatouros, Heekyoung Lee and Nathalie Eisenhardt

SUMO conjugation – a mechanistic view

DOI 10.1515/bmc-2016-0030
Received December 15, 2016; accepted February 6, 2017

Abstract: The regulation of protein fate by modification with the small ubiquitin-related modifier (SUMO) plays an essential and crucial role in most cellular pathways. Sumoylation is highly dynamic due to the opposing activities of SUMO conjugation and SUMO deconjugation. SUMO conjugation is performed by the hierarchical action of E1, E2 and E3 enzymes, while its deconjugation involves SUMO-specific proteases. In this review, we summarize and compare the mechanistic principles of how SUMO gets conjugated to its substrate. We focus on the interplay of the E1, E2 and E3 enzymes and discuss how specificity could be achieved given the limited number of conjugating enzymes and the thousands of substrates.

Keywords: E1; E2; E3 enzymes; SIM; SUMO chains; SUMO paralogs.

Introduction

Reversible posttranslational modification (PTM) with the small ubiquitin-related modifier SUMO (sumoylation) is conserved in all eukaryotes. SUMO belongs to the superfamily of ubiquitin-like (Ubl) modifiers and performs essential functions in most organisms. Sumoylation is involved in a large variety of fundamental cellular processes, including DNA replication, transcription, cell cycle regulation, DNA damage repair, chromatin organization, ribosome biogenesis, pre-mRNA splicing, nuclear trafficking, signal transduction and protein degradation (1–12). Such a plethora of functions implies the existence of multiple targets. Indeed, more than 1000 sumoylated proteins have been identified, with the numbers continuously increasing (13). Sumoylation is highly dynamic and the global SUMO proteome is constantly changing, for example, during cell cycle progression and cell differentiation, and it is drastically induced upon stress (14–17). Such stress stimuli include DNA damage, heat shock, proteasomal inhibition, viral infection or ischemic challenge. These significant rearrangements in the SUMO proteome appear to represent a versatile immediate stress response, required, for example, for DNA damage repair (2, 7, 18) or to protect the brain against focal cerebral ischemic damage (19). However, constitutively increased sumoylation has rather negative effects and correlates with resistance to cancer treatments, increased tumor metastasis and relapse (20–24). Also, several other diseases, like neurological disorders, diabetes and heart failure, were connected to defects in the SUMO system (25–28). Together, these findings point to an important role of sumoylation in maintaining cell homeostasis and it is of key importance to understand its substrate specificity and regulation.

A comprehensive analysis of diverse SUMO substrate screens revealed that different groups of proteins, including cell cycle and DNA damage repair factors, show increased sumoylation in response to stress, while the modification is removed in other groups of proteins, such as nucleosome components and transport factors (13). This points to a broad but highly regulated system performed by the counteracting activities of SUMO-conjugating enzymes and SUMO-specific proteases. It is currently unclear how these individual groups of substrates are selected.

In this review, we will discuss general features of sumoylation, with a focus on the mechanistic aspects of how SUMO conjugation is executed at the enzymatic level in *Saccharomyces cerevisiae* and mammalian cells. We will illustrate regulatory concepts occurring at different levels and discuss how substrate specificity could be achieved. SUMO deconjugation was recently reviewed in great detail (29, 30) and will only be mentioned in a general context.

Synopsis of SUMO conjugation and deconjugation

All SUMO proteins are expressed as immature precursors and need to be matured by SUMO proteases to expose

***Corresponding author: Andrea Pichler,** Department of Epigenetics, Max Planck Institute of Immunobiology and Epigenetics, Stübeweg 51, D-79108 Freiburg, Germany, e-mail: pichler@ie-freiburg.mpg.de
Chronis Fatouros, Heekyoung Lee and Nathalie Eisenhardt: Max Planck Institute of Immunobiology and Epigenetics, Department of Epigenetics, Stübeweg 51, D-79108 Freiburg, Germany

the C-terminal di-glycine (GG) motif that is critical for conjugation (Figure 1). In an ATP-consuming reaction, SUMO is then activated by adenylation, enabling it to form an energy-rich thioester bond with the catalytic cysteine of the heterodimeric E1 enzyme, composed of the Aos1/Uba2 (Sae1/Sae2) subunits (31). Next, SUMO is passed to the catalytic cysteine of the E2 conjugating enzyme (Ubc9) (Ube2I), again resulting in a thioester bond (32, 33). In the final step, SUMO is transferred to the substrate, forming an isopeptide bond with an ε-amino group of the acceptor lysine residue (34–36). While the E2 enzyme can directly interact with a SUMO consensus motif (SCM, see below) found in many substrates, this interaction is insufficient for an efficient SUMO transfer and needs to be stabilized either by additional E2 interactions or by E3 ligases. E3 ligases interact with the substrate and the charged E2 enzyme and catalyze the discharge of the thioester-bound SUMO from the E2 enzyme to the substrate. Substrates can be modified with a single SUMO moiety, multiple SUMOs or with SUMO chains. SUMO proteases reverse the sumoylation by cleaving the isopeptide bond between SUMO and its substrate, thereby defining the balance between the free and conjugated SUMO pool, as well as the dynamic steady-state levels of sumoylated substrates in the cell.

SUMO proteins

SUMO proteins share a common three-dimensional structure, characterized by a tightly packed globular fold with β-sheets wrapped around one α-helix (37) (Figure 2A). In contrast to ubiquitin, SUMO proteins bear a highly flexible N-terminal extension that contains the major site for SUMO chain formation (37). The exact site of chain linkage and the efficiency of chain assembly differ between SUMO paralogs (38).

Saccharomyces cerevisiae contains a single SUMO protein, Smt3, while mammalian cells express up to five SUMO paralogs (SUMO1–SUMO5). The Smt3 knockout is lethal in *S. cerevisiae* (www.yeastgenome.org). Surprisingly, mice deficient in SUMO1 (39, 40) and SUMO2 (41) are viable and only SUMO3 is essential for embryonic development (embryos die around embryonic day E10.5), most likely because it is the predominantly expressed SUMO isoform (41). There is considerable confusion about the SUMO2 and

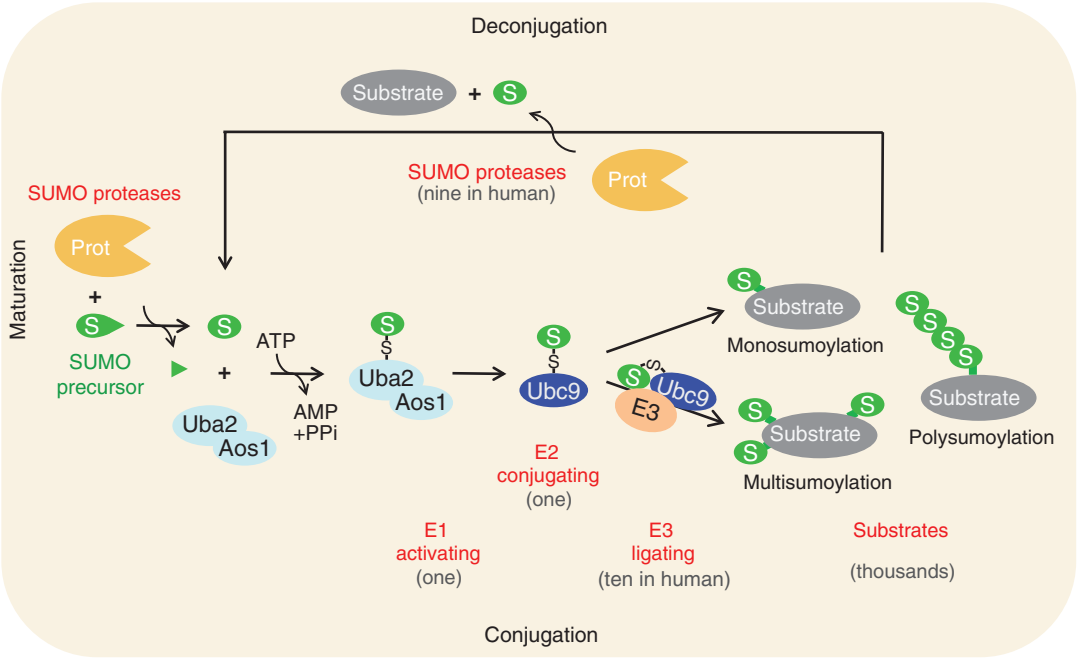


Figure 1: SUMO conjugation cycle.
The covalent attachment of SUMO to its substrates is processed by the hierarchical action of an enzymatic triad, involving E1, E2 and E3 enzymes. First, SUMO (S) is matured by SUMO specific proteases (Prot), enabling it to become activated in an ATP-consuming reaction, to form a thioester bond (-S-) with the heterodimeric E1 (Aos1/Uba2). SUMO is then transferred to the E2 (Ubc9), again resulting in a thioester bond. Finally, SUMO is conjugated directly or with the help of an E3 ligase to its substrate, forming an isopeptide bond (solid line). Substrates can be modified with a single SUMO (monosumoylation), with multiple SUMOs (multisumoylation) or with a SUMO chain (polysumoylation). Sumoylation is reversed by SUMO specific proteases that cleave SUMO from the substrate. The known number of SUMO enzymes, given in brackets, is limited, in contrast to the thousands of known substrates.

SUMO3 paralogs in the literature and in major databases. We use the original nomenclature for SUMO2 and SUMO3 as described by Saitoh and Hinchey (42) throughout this review, which might differ from some sources.

SUMO1–SUMO3 are ubiquitously expressed while expression of SUMO4 and SUMO5 is restricted to specific tissues (43–45). Importantly, the final proof that SUMO4 and SUMO5 are translated into proteins remains to be shown, especially as they were originally annotated as pseudogenes [see (46) and comment of M. Tatham in Pubmed on (45)]. SUMO1 and SUMO2/3 share less than ~50% sequence identity (Figure 2B). SUMO2 and SUMO3 are almost identical (~97% in humans) and cannot be distinguished by antibodies. Thus, they are often referred to as SUMO2/3 (42, 47).

Under physiological conditions, SUMO1 is constitutively conjugated to substrates, while all other SUMO paralogs are preferentially conjugated in response to stress (42, 45, 48).

Diverse proteomic studies showed that SUMO can be modified by different PTMs, including acetylation, phosphorylation and ubiquitination, indicating that it is itself a target for complex regulation (13). At present, only SUMO acetylation and phosphorylation have been analyzed in greater detail: SUMO (SUMO1 Lys37 or SUMO2 Lys33) acetylation interferes with binding to a SUMO interaction motif (SIM) [see below and (49)]. SUMO1 Thr76 phosphorylation appears to regulate its stability and activity in cells, although the exact molecular mechanisms remain to be resolved (50). It will be thrilling to gain further insight into these different SUMO regulatory mechanisms because such modifications are likely to have a powerful impact on interacting proteins, with severe consequences for enzymes and substrates. Additionally, such modifications may regulate cellular functions like stability or localization of SUMO proteins, as has been proposed for SUMO1 phosphorylation.

The SUMO consensus motif

Early on in the discovery of SUMO substrates, it became evident that many share a common ΨKxE (Ψ = hydrophobic residue with high preference for I or V) motif for conjugation, designated SUMO consensus motif (SCM). In recent years, several efforts have been made to study sumoylation on a global and site-specific manner by high resolution mass spectrometry (14, 15, 51–54). Reanalyzing all these available data sets (13) revealed that at physiological conditions, at least half of the SUMO substrates are modified at the minimal KxE motif, although upon stress, more lysines at non-SCM sites are modified.

SCMs directly interact with the catalytic cleft of the E2 enzyme and this interaction contributes to catalysis and to lysine selection (55, 56). However, for an efficient SUMO transfer, this interaction needs to be stabilized, either by additional binding interfaces or co-factors, or by the help of an E3 ligase. Consistently, extended and regulated variations of this SCM motif have been discovered that lead to an increase in E2 affinity and enhanced modification *in vitro*. Such an increase can either be achieved by a hydrophobic cluster N-terminal of the core SCM, termed hydrophobic cluster sumoylation motif (HCSM) (57), or by negatively charged amino-acids C-terminal of the core SCM, termed negatively charged amino-acid-dependent sumoylation motif (NDSM) (58). Additionally, a phosphorylation site C-terminal of the core SCM in a phosphorylation-dependent sumoylation motif (PDSM) can mimic negatively charged amino acids when modified (59–61). Of note, all such motifs can also be found in many non-sumoylated proteins, demonstrating that their mere presence does not necessarily define a SUMO substrate. SCM sumoylation consistently depends on the structural context, requiring extended or unstructured and exposed surface regions (62).

Non-covalent SUMO interactions

Besides covalent substrate modification, SUMO also regulates protein function in a non-covalent manner, either in its free or in its conjugated form. Such SUMO-specific protein-protein interactions can have diverse consequences on the fate of the involved proteins, like changes in their intracellular localization, protein stability or enzymatic activity (62–69).

SUMO utilizes three different binding interfaces to non-covalently interact with other proteins and accordingly, we distinguish three different classes (Figure 2). Class I interactions recognizing the SUMO interaction motif (SIM) on substrates present the best-characterized and most prominent interaction surface on SUMO. This SIM is established by a short stretch of three to four hydrophobic amino acids embedded in a β-strand that interacts with SUMO and flanking acidic regions reviewed in (70). Recent structural studies disclosed an unpredicted alleviated variation of class I SIMs with only two hydrophobic amino acids that are also placed in a central β-strand (71). Class II SUMO interactions exhibit higher affinities [~80 nM (72)] than SIM interactions [1–100 μM (73–76)]. They utilize a binding surface opposite to the class I site, thus

allowing simultaneous class I and class II SUMO binding. Two examples have been identified, the SUMO-Ubc9 backside interaction (72, 77–79) and the interaction between SUMO1 and dipeptidyl peptidase-9 (DPP9) (80). Of note, Ubc9 and DPP9 bind to a similar but not identical surface on SUMO. The class III SUMO interaction was initially discovered by the interaction of the ubiquitin E3 ligase HERC2 with SUMO1 via its ZZ type zinc-coordinating motif (81). A recent nuclear magnetic resonance (NMR) structure of the CREB-binding protein (CBP) ZZ zinc finger domain interacting with SUMO identified this third binding interface on SUMO, which is separate from the class I and class II surfaces (82). A synopsis of the three different non-covalent SUMO binding interfaces is depicted in Figure 2.

It is still difficult to predict SUMO interactions *in silico* because of the many variations. Predicted SIMs do not mandatorily bind SUMO and too few examples are identified to describe motifs for the other classes of SUMO binding.

Importantly, non-covalent SUMO interactions contribute to regulation because SUMO interactions can be selective for SUMO paralogs. Some class I SIMs demonstrate paralog specificity, probably through additional interactions adjacent to the hydrophobic core (75, 76, 83–87). Also, the class II DPP9, but not Ubc9, shows specificity for SUMO1, while the class III HERC2 displays preference for SUMO1 (80, 81).

To further increase the complexity of non-covalent SUMO-SIM interactions, both SUMO and SIMs can be dynamically modulated by PTMs to change the charge of the respective binding partner. This can either stabilize or interrupt the interaction in a regulated manner. One exciting example is the acetylation of SUMO1 at Lys37 or SUMO3 at Lys33 (SUMO2 Lys32) that controls selected SUMO-SIM interactions by neutralizing the positive charges of amino acid residues surrounding the SIM docking site (49). Moreover, introduction of a negative charge by phosphorylation of serine residues adjacent to the SIM hydrophobic core enhances the non-covalent interactions with SUMO via Lys39, His43 and Lys46 in SUMO1 and His17, Lys35 and His37 in SUMO3 (76, 86–89). A more detailed functional and structural analysis will be required to fully understand the complexity of non-covalent SUMO interactions and their regulation by PTMs.

The E1 activating enzyme

The sole SUMO E1 enzyme has to execute several functions that are essential for SUMO conjugation. It must select SUMO among the ubiquitin-related modifiers (ubiquitin, Nedd8, SUMO, etc.) and thus provides specificity for SUMO conjugation. Then, it activates the C-terminal glycine of the mature SUMO by adenylation in an ATP-consuming reaction. This enables the attack by the conserved catalytic cysteine on the E1 enzyme to form a highly reactive SUMO~E1 thioester bond. Finally, the E1 recognizes the SUMO-specific E2 (Ubc9) and enforces the SUMO transfer to the E2 enzyme (32, 90, 91).

The SUMO E1 enzyme was originally discovered in *S. cerevisiae* based on sequence similarity to the ubiquitin E1 enzyme (Uba1) (92). In contrast to Uba1, the SUMO E1 enzyme is a heterodimer composed of the Aos1 and Uba2 subunits (also referred to as Sae1 and Sae2 in mammals). Structural analysis revealed that Uba2 contains three domains: an adenylation domain (adenylation active site), a catalytic domain (Cys 173 responsible for thioester bond formation in the human Uba2) and a Ubl domain with structural similarity to ubiquitin and other Ubl modifiers. The mammalian Uba2 subunit contains a C-terminal extension (32) with a predicted nuclear localization signal (NLS) (93) and two SIMs (73, 90). While this region is dispensable for E1 function *in vitro* and in *S. cerevisiae in vivo* (32, 93), it probably has regulatory functions specific to mammalian cells.

E1 regulation

As expected, the regulation of E1 activity results in global changes in the highly dynamic SUMO proteome. In general, E1 regulatory mechanisms demonstrate a quick response in several systems involving environmental changes. Low levels of reactive oxygen species (e.g. H_2O_2) and anticancer drugs used for the treatment of acute myeloid leukemia induce the formation of a disulfide bridge between the catalytic Cys residues of the E1 and the E2 enzymes. This leads to the transient inactivation of both enzymes and the subsequent desumoylation of most cellular substrates (94, 95). Interestingly, under the same conditions, the overall ubiquitination was not affected (94).

PTMs were also shown to control E1 activity. Sumoylation of the human Uba2 subunit at Lys 236 neither influences SUMO adenylation nor E1~SUMO thioester formation but impairs its interaction with the E2 enzyme. Consistently, Uba2 sumoylation is decreased upon heat shock, which correlates with increased global sumoylation (96).

Another regulatory mechanism has been described for the Cleo adenovirus Gam1 protein that targets the E1 enzyme for proteasomal degradation following viral infection. Gam1 functions as a substrate adaptor, recruiting

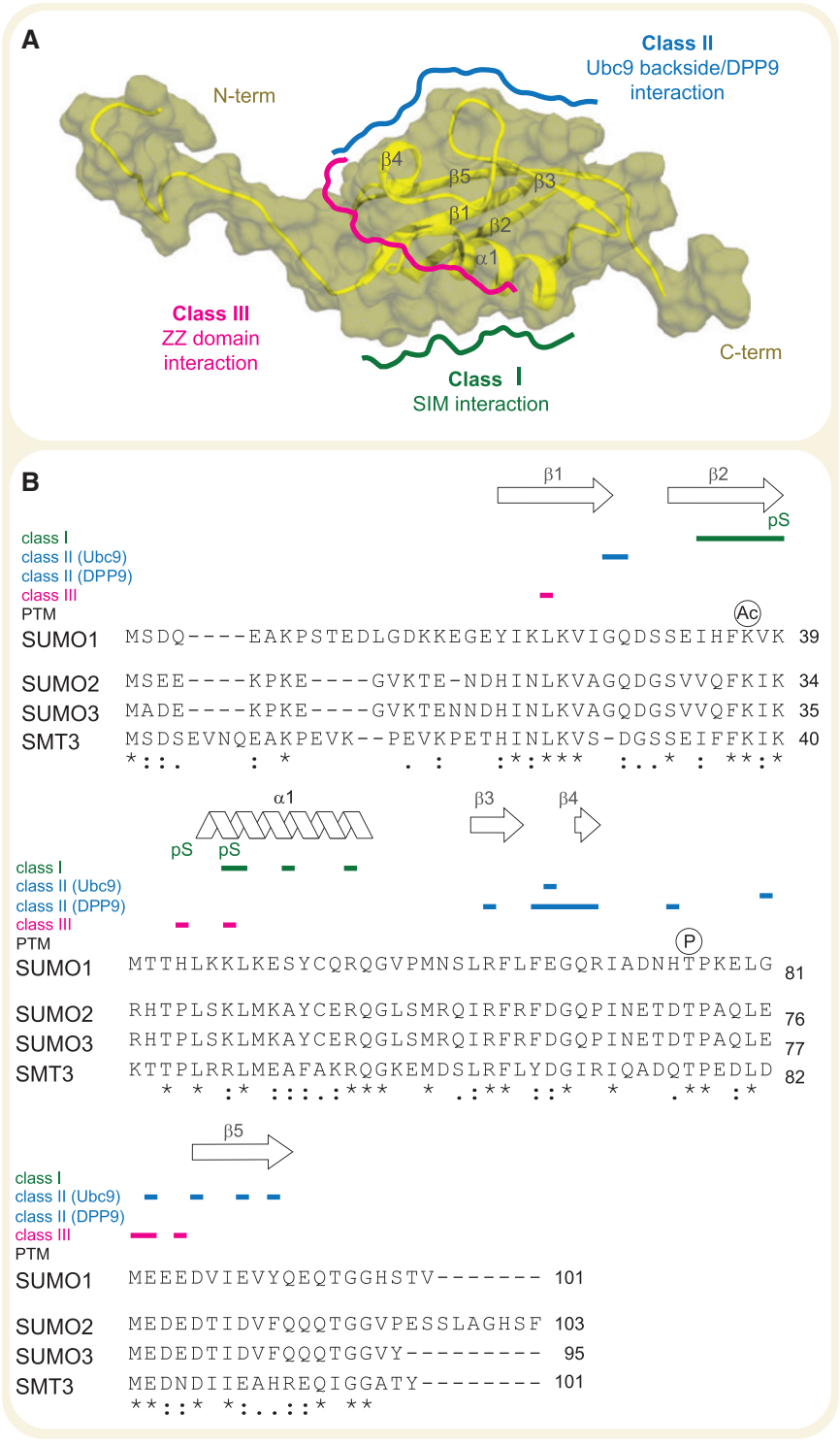


Figure 2: Non-covalent SUMO binding interfaces.
(A) Three classes of SUMO interactions are defined by distinct binding interfaces. The surface structure of SUMO1 is shown [yellow, based on 1A5R, (37)] with the classical class I SIM surface [residues I34-K39, K46, L47, S50, R54, (76, 209)] indicated in green, class II SUMO binding in blue [backside of Ubc9 involves G28, Q29, E67, G81, E83, D86, E89, Y91 (72, 77) and DPP9 R63, F66-R70, H75; (80)] and class III ZZ domain interaction in magenta [residues L24, H43, K46, M82, E83, E85; (82)]. N-term and C-term indicate the N- and C-termini of the protein. Secondary structure elements (α: alpha-helix, β: beta-sheet) are labeled and numbered. (B) A sequence alignment of SUMO1, 2, 3 and Smt3 is shown and the structural features are highlighted. Amino acids involved in the particular interactions based on SUMO1 are marked in the same colors as in (A) and residues that are targets of modifications are indicated as Ac for acetylation and P for phosphorylation. pS demonstrates phospho-SIM interactions.

the SUMO E1 to the Cullin-RING ubiquitin ligase complex Cul2/5-EloB/C-Roc1 for its degradation (97).

Because of its key regulatory role, the SUMO E1 represents an interesting target for drug development. Loss of SUMO conjugation delays tumor progression in cellular model systems and xenograft models (98). Concordantly, E1 downregulation was recently shown to inhibit colorectal cancer stem cell maintenance and self-renewal (21). Therefore, approaches to target the SUMO system, particularly by interfering in a dose-dependent manner not only with the E1 but also the E2 enzymatic activities, are increasingly discussed and already in experimental phases (21, 22, 99, 100).

The E2 conjugating enzyme

The central enzyme in SUMO conjugation is the sole E2 enzyme Ubc9 (Ube2I). It interacts with the E1 to accept SUMO and forms a SUMO~Ubc9 thioester bond. Subsequently, the charged E2 interacts with the substrate and usually with an E3, and SUMO is transferred to the substrate. Hence, the E2 enzyme possesses binding interfaces for the E1, the substrate, an E3 and also for SUMO, as summarized in Figure 3.

Structurally, all Ubc9 orthologs share the same Ubc fold, which is highly similar to other E2 enzymes of the ubiquitin pathway (101, 102). The Ubc domain has a compact ellipsoid shape and consists of four α-helices and one anti-parallel β-sheet formed by four β-strands. The key catalytic residue within this ~150 amino acid domain is Cys 93, which forms a thioester linkage with the SUMO C-terminus (103).

Because of the essential function of the E2 enzyme in SUMO conjugation, it is not surprising that a knockout is lethal in most eukaryotes (104–109). Mice deficient in Ubc9 die early in development, prior to E7.5 (104).

E2-substrate interactions

Compared to its ubiquitin homologs, Ubc9 is unique in its ability to recognize a SCM and thus selects the lysine for modification. However, the Ubc9 residues in direct contact with the SCM contribute more to catalysis than to stable substrate binding (55, 56). The substrate-E2~SUMO complex needs to be stabilized to allow an efficient SUMO transfer. This can be achieved by additional E2-substrate binding interfaces, co-factors or E3 ligases.

Astonishingly, in the last decade, several mechanisms were discovered that stabilize the substrate-E2 interaction and enhance sumoylation *in vitro* (Figure 4).

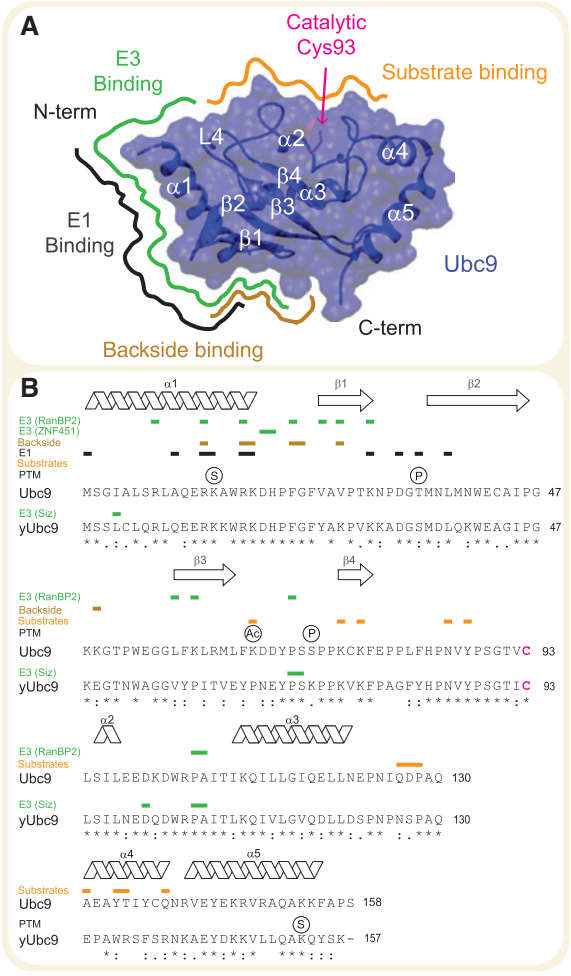


Figure 3: The multiplicity of E2 interactions.
(A) An overview of the indicated binding interfaces on a surface structure of Ubc9 [blue, based on 3UIN, (193)] is shown. The N-terminus (N-term) of Ubc9 binds the E1 (black line) and selected E3s (green line). The E2 catalytic cleft around Cys93 directly recognizes a SCM in substrates, but in addition various E2-substrate binding interfaces have been mapped near to the catalytic cleft (orange line, see also Figure 4). The backside of Ubc9, opposite to its catalytic center, binds SUMO (brown line) and this surface partially overlaps with the E1 and selected E3 interaction sites. C-term indicates the C-terminal end of the protein, secondary structure elements (α: alpha helix, β: beta sheet) are labeled and numbered. (B) A sequence alignment of human and yeast Ubc9 is shown with structural features indicated. Amino acids involved in the individual interactions are shown in the same color as in (A). Residues that are targets of modifications are indicated as Ac for acetylation, S for sumoylation and P for phosphorylation.

– Constitutive and regulated SCM extensions. As mentioned above, three types of short extensions of SCMs (see above) are proposed to increase the interaction with the E2 enzyme, a HCSM (57), a NDSM (58) and a PSDM that mimics negatively charged amino acids upon modification (59–61).

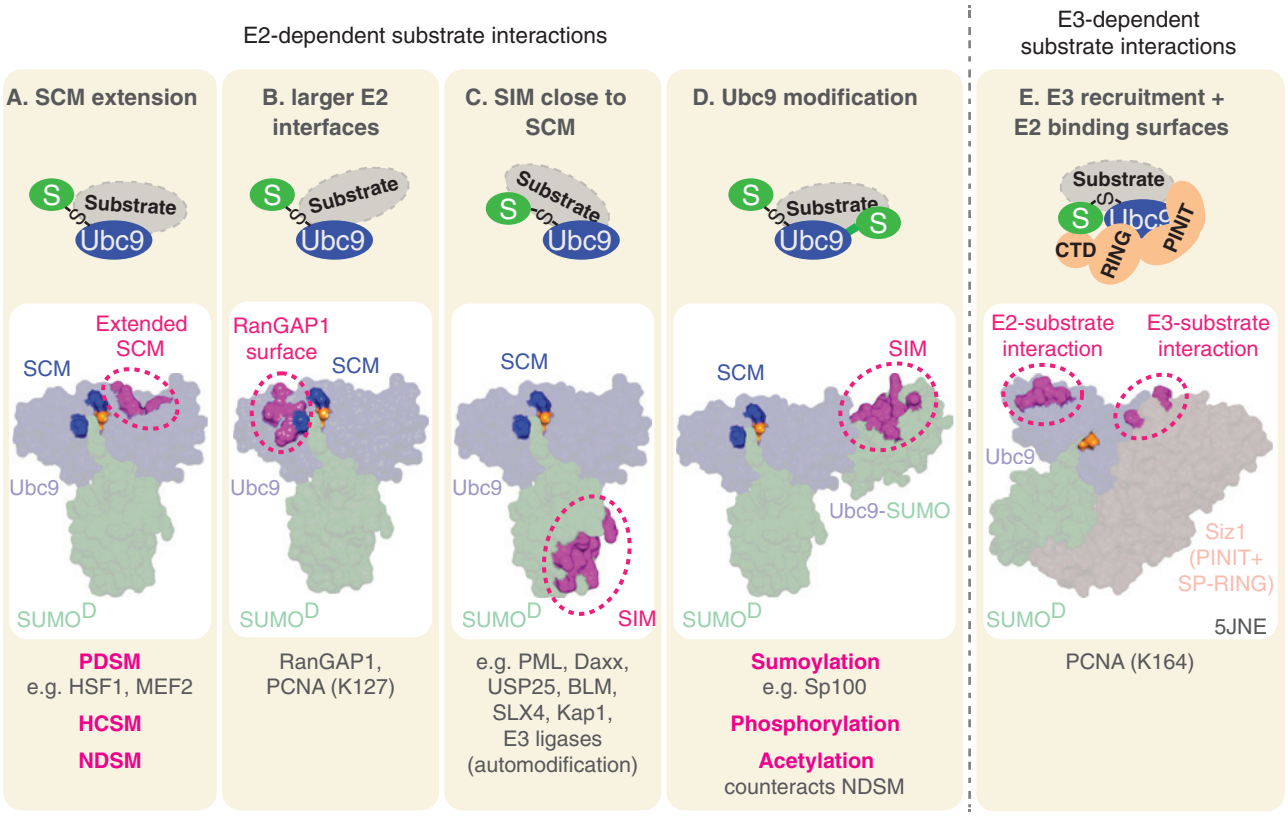


Figure 4: E2 and E3 dependent substrate interactions.
The E2 catalytic cleft directly recognizes a SUMO consensus motif (SCM) in a substrate. This interaction is inefficient for substrate selection and needs to be stabilized by additional binding interfaces for efficient modification. This can be provided by additional E2 binding interfaces (A-D) or by the presence of an E3 (E). The comparison of various substrate interaction modes in the absence and presence of an E3 is shown as simplified schematic cartoons. The surface structures and example substrates are indicated. In the cartoons, substrates are in grey, SUMO in green, and Ubc9 in blue. Thioester-bonds are indicated as -S- and isopeptide-bonds as solid lines. The surface structures highlight the substrate interfaces (magenta) on the E2 and, where applicable, on SUMO^D, a SUMO conjugated to Ubc9 or the E3. For clarity, the substrate itself is not shown. Substrate binding patches are modelled on surface structures of a SUMO^D loaded Ubc9 mimic [3UIN, (193)] and on SUMO^D loaded Ubc9 in a complex with an E3 [5JNE, (71)]. Structural data for a charged Ubc9 are only available in the presence of an E3 that orient the SUMO^D in a closed conformation. To date, there is no evidence that E2-dependent sumoylation also involves a closed conformation and hence, in the cartoons, the closed conformation is only shown in the presence of the E3. Ubc9 Cys93 is depicted in orange, SCM-E2 interactions are highlighted in blue and additional substrate interfaces are shown in magenta in the structural models. (A) Three distinct SCM extensions have been described to increase E2-substrate affinity: PDSM (phosphorylation-dependent sumoylation motif), HCSM (hydrophobic cluster sumoylation motif) and NDSM (negatively charged amino acid-dependent sumoylation motif). Residues involved in the interaction with a substrate displaying a phosphorylation site adjacent to the SCM are shown, as described for HSF1 and MEF2 (59, 60). (B) RanGAP1 employs a lager surface adjacent to the SCM to interact with Ubc9 (55). Interestingly, PCNA uses a similar interface to interact with Ubc9 (71). (C) Some SUMO substrates depend on a SIM close to a SCM for efficient modification (69, 71, 75, 83, 84, 113–117, 125). (D) Posttranslational Ubc9 modifications, like sumoylation, acetylation or phosphorylation, can also regulate substrate specificity. A Ubc9 sumoylation that can enhance the affinity to selected SIM-containing proteins is depicted (sumoylated Ubc9 (2VRR) (69), superimposed with the SUMO^D charged Ubc9 mimic, according to 1Z5S (58). (E) E3-dependent substrate interactions are shown for PCNA, involving binding interfaces with the E2 and the E3 [5JNE, (71)].

– Larger interfaces near the SCM. RanGAP1 is unique in its ability to stably interact with the E2 and it is probably the most efficient *in vitro* SUMO substrate in the absence of an E3 ligase (110). Accordingly, RanGAP1 represents the most abundant sumo(1)ylated protein in many cell types, even though a very low deconjugation rate also contributes to this phenotype (111). Structural analysis revealed an additional binding interface close to the SCM that is required for stable E2 binding and efficient modification (55). Interestingly, PCNA interacts with Ubc9 via a surface similar to that recognized by RanGAP1, and PCNA also gets modified in an E3-independent manner at Lys 127 *in vivo* and *in vitro* (12, 71, 112). Of note, the Ubc9-PCNA interface was identified in the presence of its E3 ligase (71) but it is very likely that a similar

E2-substrate interface plays a role in E3-independent PCNA modification.
- A SIM close to the SCM. Such a SIM can be important for efficient substrate modification by stabilizing the interaction with the SUMO-charged E2. Examples are well-characterized SUMO substrates like the promyelocytic leukemia gene product PML, the death-domain-associated protein-6 Daxx, the ubiquitin-specific protease Usp25 and the Bloom's syndrome helicase BLM (69, 84, 113–115). As selected SIMs display SUMO paralog specificity, such motifs can dictate paralog-specific substrate modification (84, 114). In contrast to the other activities that stabilize the E2 interaction with the substrate, these SIM-mediated interactions with the charged E2 might position the donor SUMO ($SUMO^D$) in a closed conformation, similar to that demonstrated for *bona fide* E3 ligases (discussed below). Intriguingly, this could explain the efficient cis-modification activities (automodifcations) of *bona fide* E3 ligases and diverse SIM-containing proteins like KAP1 (116) or Slx4 (117). However, detailed biochemical, and preferably structural evidence, is required to clearly distinguish between the SIM-dependent enhancement in catalysis (as it is the case for *bona fide* E3 ligases), as opposed to enhancement of substrate affinity.
- Posttranslational Ubc9 modifications that regulate substrate interaction. N-terminal Ubc9 sumoylation in mammalian cells can enhance the affinity to substrates with a SIM in close distance to the SCM, as we have shown for the transcriptional regulator Sp100 (69). A related mechanism was recently proposed for Ubc9 phosphorylation, although the molecular details remain to be shown (118). Interestingly, Ubc9 acetylation regulates substrate selection by exclusions, as it removes the positive charge on Ubc9 required for the interaction with NDSM-containing SUMO substrates (119).

It is of key importance to mention that until now, it is unclear whether these different mechanisms are indeed sufficient for substrate sumoylation *in vivo* or whether the help of E3 ligases is still required. Nevertheless, what becomes intriguingly evident from all these examples is that the SUMO E2 enzyme has a greater role in substrate selection than is described for any other E2 enzyme of the Ubl system.

SUMO-E2 backside interaction

Besides substrate-binding interfaces, another important regulatory E2 interface is its backside, opposite to the catalytic cysteine. This interface interacts with SUMO (class II SUMO interaction, see above) (72, 77–79, 120, 121) with an affinity approximately 1000-fold higher than that found for ubiquitin and its cognate E2s [SUMO and Ubc9 interact with a K_d of ~80 nM (72), ubiquitin and UbcH5c with a K_d of ~300 μM (122) or ubiquitin and Mms2 with a K_d of ~100 μM (123)]. Interest in this interface is increasing as it partially overlaps with the E2-E1 interface (78), plays an important role in direct or indirect E2-E3 interactions (71, 75, 83, 124, 125) and in E2-E2 interactions (68). It also has key functions in SUMO chain formation (68, 72, 77, 83), as discussed in greater detail in the respective following sections.

E2 regulation

As Ubc9 is the only known SUMO E2, and due to its essential functions in SUMO conjugation, it is not surprising that regulation of its catalytic activity has effects on global cellular sumoylation, comparable to the E1. However, regulating specific substrate, or E3-binding interfaces, are expected to only target subgroups of substrates.

General regulators of E2 functions include regulators of its expression level or of its catalytic activity. For example, the viral HPVE6 protein or infection with the bacterium *Listeria monocytogenes* were shown to reduce cellular Ubc9 expression levels (126, 127), while various microRNAs (miRNAs) expressed in cancer cells are proposed to upregulate Ubc9 expression. Of note, Ubc9 overexpression often correlates with different types of cancer [e.g. (128–131)]. More recently, it was shown that miRNA-30a controls Ubc9 levels in human subcutaneous adipocytes, with consequences for their mitochondrial activity (132). Another way to directly regulate the E2 catalytic activity is the previously-discussed transient disulfide bridge formed between the catalytic cysteines of the E1 and the E2 enzymes (94, 95). Not surprisingly, PTMs also represent an additional strategy to regulate E2 function. E2 phosporylation at Ser71 or Thr35 appears to promote Ubc9~SUMO thioester formation (50, 133). In *S. cerevisiae*, C-terminal E2 sumoylation at Lys153 drastically impairs its catalytic activity *in vitro*, probably by stably binding to the E2 backside and thereby preventing E1 accessibility. This is supported by the crystal structure of Smt3 bound to the backside of Ubc9, wherein the C-terminus of SUMO is in close proximity to Ubc9 Lys153 (78). Mammalian Ubc9 is modified at Lys14 on the opposite side of Ubc9 and can, for steric reasons, not fall into the backside position (69). However, this C-terminal E2 modification in yeast was able to turn the inactive E2 into a cofactor,

accelerating SUMO chain formation [(68), see also below and Figure 7].

In clear contrast to general regulators of Ubc9 function, the above-mentioned E2 modifications that interfere with specific substrate interactions like acetylation, N-terminal sumoylation in mammals (currently, there is no evidence that C-terminal Ubc9 sumoylation can bind to substrates) and phosphorylation display a more selective form of regulation, as these modifications only affect a subgroup of SUMO substrates (50, 72, 118, 119).

Another group of Ubc9 regulatory proteins include some members of SUMO-like proteins with important functions in DNA repair and the maintenance of genome stability. Two types of such SUMO-like proteins have been described, the RENi protein family (Rad60 in fission yeast, Esc2 in baker's yeast and NIP45 in mammals) and the ubiquitin protease USP1/UAF1 (134, 135). All of these proteins share two tandem SUMO-like domains (SLD1 and SLD2). Structural and biochemical analysis of RENi SLDs revealed that these domains are unable to bind to class I SIMs but SLD2 interacts with the backside of Ubc9 by mimicking the β-sheet required for this class II SUMO interaction (136, 137). Of note, RENi proteins also contain a conserved SIM in their N-termini and bind to SUMO, but they are themselves inefficient sumoylation substrates (138–140). In contrast to RENi proteins, the SLD2 domain of UAF1 directly binds to a class I SIM, and this interaction is required for its activity, but does not regulate E2 functions (135).

E3 ligating enzymes

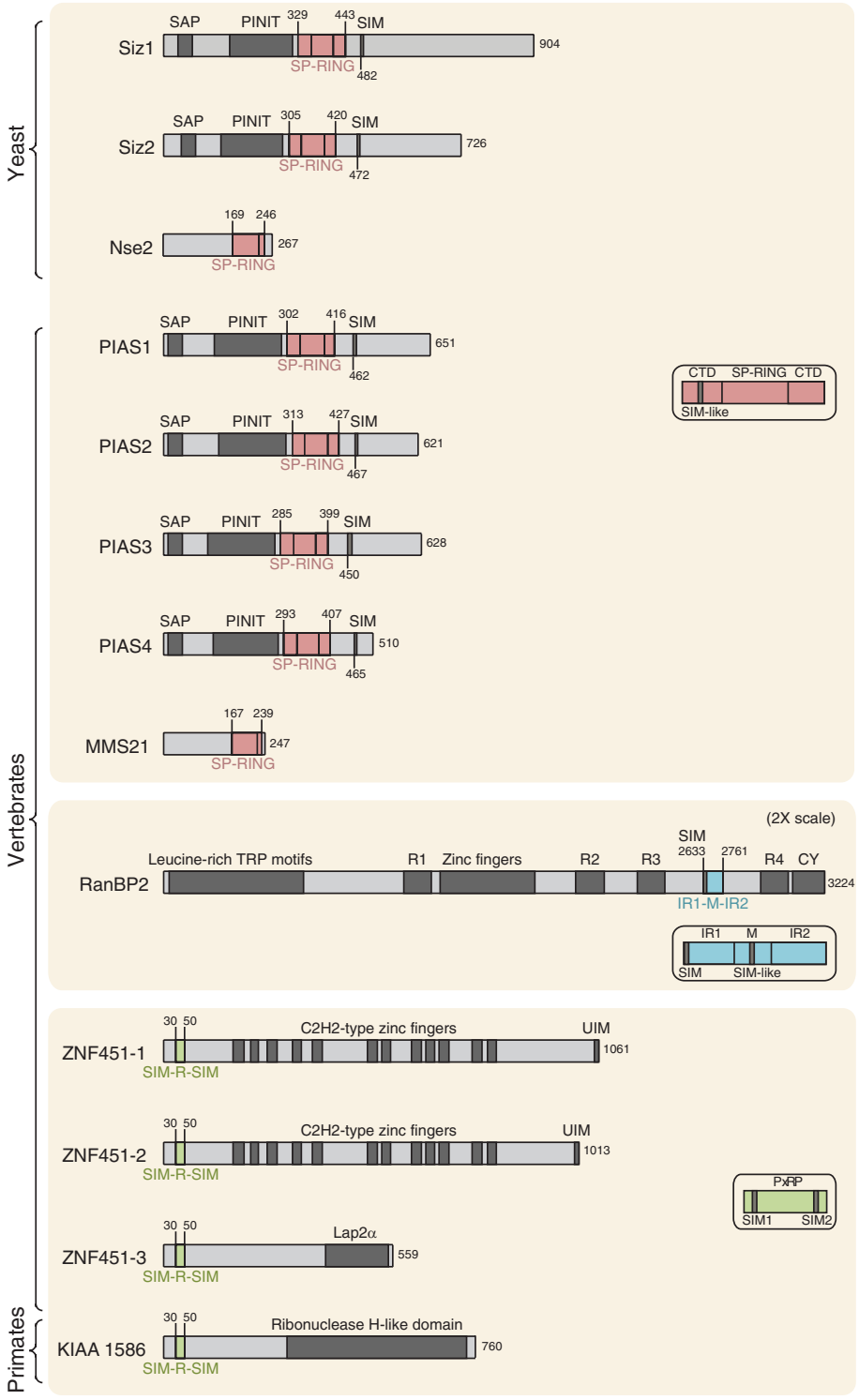
E3 ligases catalyze the transfer of SUMO from the charged E2 enzyme (Ubc9~SUMO) to the substrate. Hence, they interact with Ubc9~SUMO and the substrate to bring them in close proximity. It has only recently become evident that all *bona fide* SUMO E3 ligases align $SUMO^D$ (the thioester-bound SUMO that gets transferred to the substrate) in a nearly identical, highly reactive closed conformation, with an optimal orientation for the nucleophilic attack by the incoming lysine on the substrate. This conformation is required for the efficient discharge of Ubc9 and subsequent substrate sumoylation (71, 75, 83, 125). Catalysts are recycled in the reaction, allowing many rounds of substrate modification by a single enzyme. Therefore, a hallmark of an E3 ligase is its ability to function at substoichiometric concentrations relative to its substrate. Usually, catalysts are not consumed in the reaction, but all known SUMO and ubiquitin E3 ligases are highly automodified. Hence, the automodification (cis-reaction) needs to be clearly distinguished from substrate modification (trans-reaction). Consequently, describing *bona fide* SUMO E3 ligases requires a detailed biochemical analysis, preferably combined with structural analysis, to define substoichiometric trans-reactions and the awareness of how the E3 discharges $SUMO^D$ from the E2 for its transfer to the substrate. Enhancement of substrate sumoylation in cells does not necessarily equate with E3 ligase function because indirect effects like regulatory co-factors or inhibition of SUMO proteases can also lead to the same outcome. In the literature, several proteins have been proposed to be SUMO E3 ligases, but comprehensive biochemical and structural analyses that allow insights into their mode of SUMO catalysis are currently only provided for three classes: the SP-RING (Siz/Pias) family, RanBP2 and the ZNF451 family. For all other proposed SUMO E3 ligases, we must wait for additional knowledge that reveals the molecular basis of their sumoylation-enhancing activities.

Classes of *bona fide* SUMO E3 ligases

SP-RING family

The SP-RING family was the first discovered family of SUMO E3 ligases. The founding members were Siz1 and Siz2 in *S. cerevisiae* that share a highly conserved ubiquitin RING E3-related structure with protein inhibitor of activated STAT (Pias) proteins in higher eukaryotes (141). Indeed, shortly thereafter, several studies confirmed that mammalian Pias proteins display SUMO E3 ligase activity (141–146). In addition, the methyl methanesulphonate-sensitivity protein Mms21/Nse2, a subunit of the Smc5/6 complex, bears a SP-RING but is otherwise unrelated to Siz/Pias proteins (147–149). To date, the SP-RING family is the only SUMO E3 ligase family evolutionarily conserved from yeast to human. As depicted in Figure 5, Siz1, Siz2 and Nse2 represent the SP-RING family members in *S. cerevisiae*, while Pias1, its splice variant Pias2, Pias3, Pias4 and Mms21 represent the vertebrate homologs.

All SP-RING E3 ligases have a C3HC4 RING domain and an adjacent SP C-terminal domain (SP-CTD). The SP-RING is highly similar to ubiquitin RING domains (141), as confirmed by structural analysis, but differs by coordinating only one Zn^{2+} ion instead of two, as seen in its ubiquitin counterparts (150). Siz and Pias proteins also share an N-terminal scaffold attachment factor A/B/acinus/PIAS (SAP) domain involved in structure- or sequence-specific DNA binding, a PINIT (Pro-Ile-Asn-Ile-Thr) motif



that interacts with PCNA, and a SUMO-interacting motif (SIM) [Figure 5 and reviewed in refs. (1, 150–152)].

Neither yeast nor mammalian SP-RING proteins are essential, and available single Pias knockouts only show mild phenotypes (141, 153–156). However, Pias1$^{-/-}$ and Pias4$^{-/-}$ double knockout embryos die before day E11.5, although it is unclear if this relates to their SUMO E3 ligase activities (157). In general, SP-RING proteins have key functions in basically all SUMO-associated pathways (152), including DNA repair (148, 158–161), cell cycle (141, 162, 163), apoptosis (162), cell migration and invasion (164, 165), oxidative stress response (166), transcriptional

Figure 5: Three classes of *bona fide* SUMO E3 ligases.
The SP-RING family is conserved from yeast to human and consists of Siz1, Siz2 and Nse2 in yeast and Pias1-4 and MMS21 in vertebrates. The common motif required for E3 activity is the SP-RING and its flanking regions that form the CTD domain (pastel red) and possess an N-terminal SIM-like motif. All Pias family members also share a SAP domain, a PINIT motif and a SIM. Nse2 and MMS21 only share the SP-RING and the C-terminal part of the CTD domain but are otherwise different. The RanBP2 E3 ligase activity was mapped to a vertebrate-specific region demonstrating two internal repeats separated by a middle region, IR1-M-IR2 (pastel blue). IR1 contains a classical SIM and the M region also binds SUMO but this interaction is not yet mapped. In addition, RanBP2 displays an N-terminal leucine-rich TRP repeat, four RanGTPase binding sites (R1-R4), eight tandem zinc fingers, multiple FG repeats (not indicated) and a cyclophilin-like (CY) domain. The ZNF451 family consists of four members, all sharing the N-terminal catalytic tandem SIM region (pastel green). ZNF451-1 to ZNF451-3 are differentially expressed from a single vertebrate specific gene locus. ZNF451-1 and ZNF451-2 differ only in one small exon. ZNF451-3, and the primate specific KIAA1586, only share the tandem SIM region with its family members but are otherwise unrelated. ZNF451-3 displays a predicted Lap2α domain and KIAA1586 has an ribonuclease H-like domain. Enlargements of the different catalytic domains are shown as insets.

regulation (87, 143–145, 167–170), reviewed in (171, 172), inflammation and immunity (173–177), infection (153) and adipogenesis (178).

RanBP2/Nup358

The second class of SUMO E3 ligases consists of the Ran-binding protein 2 (RanBP2), which is not homologous to other known E3 ligases of the Ubl family. Initially, an internal repeat region (IR1 and IR2) separated by a short linker (M) was mapped to accelerate the transfer of SUMO to a substrate (110, 124, 125, 179). RanBP2 is a large 358-kDa core component of the cytoplasmic filaments of nuclear pore complexes (NPCs) with key functions in nuclear transport and mitosis. It contains an N-terminal leucine-rich domain that anchors it to the NPCs, four Ran GTPase binding sites, eight tandem zinc fingers, two internal repeats displaying the SUMO E3 ligase domain IR1-M-IR2, multiple FG repeats, the binding sites for transport receptors and a cyclophilin-like domain (Figure 5) (180, 181).

The E3 ligase domain of RanBP2 is conserved in human, mouse, bovine, chicken and frog, but absent in worm and fly. Interestingly, this correlates with an open mitosis in higher eukaryotic cells (182). Indeed, key functions for RanBP2's E3 ligase activity were found in mitosis. During mitosis, RanBP2 is largely soluble and a fraction was found in a complex with sumoylated RanGAP1 at kinetochores and the mitotic spindle (183–185). While RanBP2 knockouts in mice are embryonically lethal (186), reduced RanBP2 expression levels already resulted in severe aneuploidy, caused by the formation of anaphase bridges and chromosomal segregation defects (187). Surprisingly, transport defects were not observed, although exact transport rates were not compared (187). The similarity of the phenotype to Topoisomerase (Topo) IIα inhibition (188) led to the discovery of Topo IIα as a RanBP2-dependent SUMO substrate dependent on sumoylation for its localization to the inner centromere on mitotic chromosomes (187). Astonishingly, ectopic expression of RanBP2's SUMO E3 ligase region (RanBP2ΔFG 2553–2838, containing IR1-M-IR2 plus flanking regions) was sufficient to restore Topo IIα sumoylation, its mitotic localization and to correct anaphase bridge formation (187).

The ZNF451 Family

The ZNF451 family was only recently discovered and is unique in its high specificity for the SUMO2/3 paralogs. Like RanBP2, the ZNF451 family is vertebrate-specific and executes SUMO catalysis via another unexpected mechanism, unprecedented in the Ubl field. ZNF451 family members depend on a tandem SIM and its inter-SIM PxRP motif to discharge SUMO~Ubc9 (75, 83).

The ZNF451 family is mostly uncharacterized. The human ZNF451 gene locus encodes three isoforms, all sharing an identical N-terminal tandem-SIM region up to amino acid 63 (Figure 5). Isoform 1 of ZNF451 (ZNF451-1) is 1061 amino acids in size and, in addition, includes a coiled coil region, followed by 12 C2H2 zinc-finger domains and a C-terminal ubiquitin-interacting motif (UIM). Isoform 2 (ZNF451-2) is very similar to isoform 1, lacking only one exon removing amino acids 870–917. Isoform 3 (ZNF451-3) employs a large exon following the tandem SIM region encoding a sequence distinct to ZNF451-1 and ZNF451-2 with an annotated Lap2α domain (189). A primate-specific gene encoding for the uncharacterized KIAA1586 protein is situated adjacent to the ZNF451 gene locus. KIAA1586 represents an additional member of the ZNF451 family, sharing a nearly identical N-terminal catalytic tandem-SIM domain with only one amino acid substitution (83). Like ZNF451-3, KIAA1586 is otherwise unrelated to ZNF451-1 and ZNF451-2 with an annotated ribonuclease H-like domain. ZNF451-1, ZNF451-3, KIAA 1586 and the minimal catalytic tandem-SIM region are able to

extend SUMO chains *in vitro* (83) and all ZNF451 family members were found to be highly modified SUMO2 substrates in several cellular screens (15, 54).

The biological role of the ZNF451 family members is largely unexplored. What we know so far is that ZNF451-1 functions as a transcriptional regulator that partially locates to PML bodies (190). ZNF451-1 itself has no intrinsic transcriptional activity, but it was shown to interact with the androgen receptor and Smad3/4 to co-regulate their respective target genes (190, 191). At least some of these functions are independent of its E3 ligase activity, as SIM mutations generally did not result in significant effects (191). ZNF451-1 cooperates with RNF4 to regulate endogenous PML levels, which limits the cellular PML body numbers. Biochemical analyses revealed PML as the first *in vitro* substrate identified for ZNF451-1's SUMO E3 ligase activity (192). Further *in vitro* and *in vivo* analyses indicated key functions for the ZNF451 family members in the SUMO2/3 chain assembly, concordant with an important role for the ZNF451 family in stress-induced sumoylation, for example, upon proteasome inhibition and in the DNA damage response (83).

Insights into E3-dependent catalysis

To understand the concept of the E3 ligase function, the awareness of how it interacts with the charged E2 enzyme to catalyze the SUMO transfer is of key importance. The final proof of E3 interactions with the SUMO^{D}-charged E2 come from structural studies that are usually based on extensive preceding functional analysis. The first crystal structure of a SUMO E3 ligase was solved for the minimal catalytic region of RanBP2 interacting with a charged Ubc9 mimic (125). As this structure disclosed fundamental insights into E3 ligase catalysis, we start this section with RanBP2 and continue in the order of available structural data. A comparison of how the different E3 ligase classes interact with the charged Ubc9 is provided in Figure 6.

The SUMO E3 ligase region of RanBP2 consists of two similar internal repeats (IR1 and IR2) separated by a short linker region (M) (110). The minimal catalytic fragment requires one IR flanked by the M region (IR1-M or M-IR2), as each are sufficient to bind Ubc9 and SUMO and enhance substrate sumoylation at substoichiometric concentrations *in vitro* (110, 124, 125, 179). IR1-M is more efficient in sumoylation *in vitro* and displays a higher affinity to Ubc9 than M-IR2 (124, 179, 193). Biochemical and structural analysis of the IR1+M domain indicated that it wraps around Ubc9 with several contacts reaching from the N-terminus to the backside of Ubc9 (124, 125, 179). Excitingly, the crystal structure uncovered, for the first time, that a SIM in IR1 orients the donor SUMO1^{D} in a so-called 'closed conformation' that represents an optimal orientation for the nucleophilic attack of the incoming substrate lysine. This interaction is functionally required for efficient catalysis (125). However, in the cellular context, a significant fraction of RanBP2 IR1 binds to sumoylated RanGAP1 in a complex with Ubc9. This scaffold shifts the E3 activity to the less-active M-IR2 region (193, 194). M-IR2 binds exclusively to SUMO1 via a not-yet-mapped SUMO-SIM interaction, explaining the SUMO1 specificity of the larger RanBP2-S1*RanGAP1-Ubc9 complex (179, 193). We also observed SUMO1 specificity with the IR1-M fragment *in vitro* (195), while the larger fragments can conjugate SUMO1 and SUMO2/3 paralogs, albeit with a preference for SUMO1 (83, 125, 179, 193). Of note, in HeLa cells, RanBP2 can be depleted with RanGAP1 antibodies (110), indicating that almost all of RanBP2 is bound to RanGAP1. However, neuronal cells display significantly reduced RanGAP1 levels upon differentiation (196), presumably making the RanBP2 IR1 region accessible. It would be intriguing to compare RanBP2's E3 ligase activity in differentiated vs. non-differentiated cells and study the RanBP2-RanGAP1 ratio in correlation with E3 activity in different cell types. Such experiments would allow insights into RanBP2's physiological E3 ligase activity and its regulation.

The second crystal structure of a SUMO E3 ligase interacting with a charged E2 mimic was solved for ZNF451's tandem SIM region (75). All ZNF451 family members execute catalysis via these tandem-SIMs, and both SIMs are essential for SUMO E3 activity (75, 83). Biochemical and structural analyses indicated that one SIM positions the Ubc9-linked SUMO^{D}, again resulting in a closed conformation almost identical to RanBP2 (75, 83). The second SIM binds a scaffold SUMO on the backside of Ubc9 (SUMO^{B}) to stabilize the catalytic intermediate, resulting in an approximately 10 times tighter interaction required for efficient substrate modification (75, 83). The crystal structure disclosed that the interaction is mediated by positioning the β-strand of the first SIM antiparallel to the β-sheet of SUMO^{D}, while the β-strand of the second SIM is in a parallel orientation to the β-sheet of SUMO^{B}. Furthermore, a PxRP motif from the inter-SIM region wedges into the interface between the two SUMO molecules and establishes critical contacts between ZNF451 Arg40 and Ubc9 Asp19 and His20, representing the only contacts with the E2 enzyme (75). Mutating these critical amino acids, or changing the length of

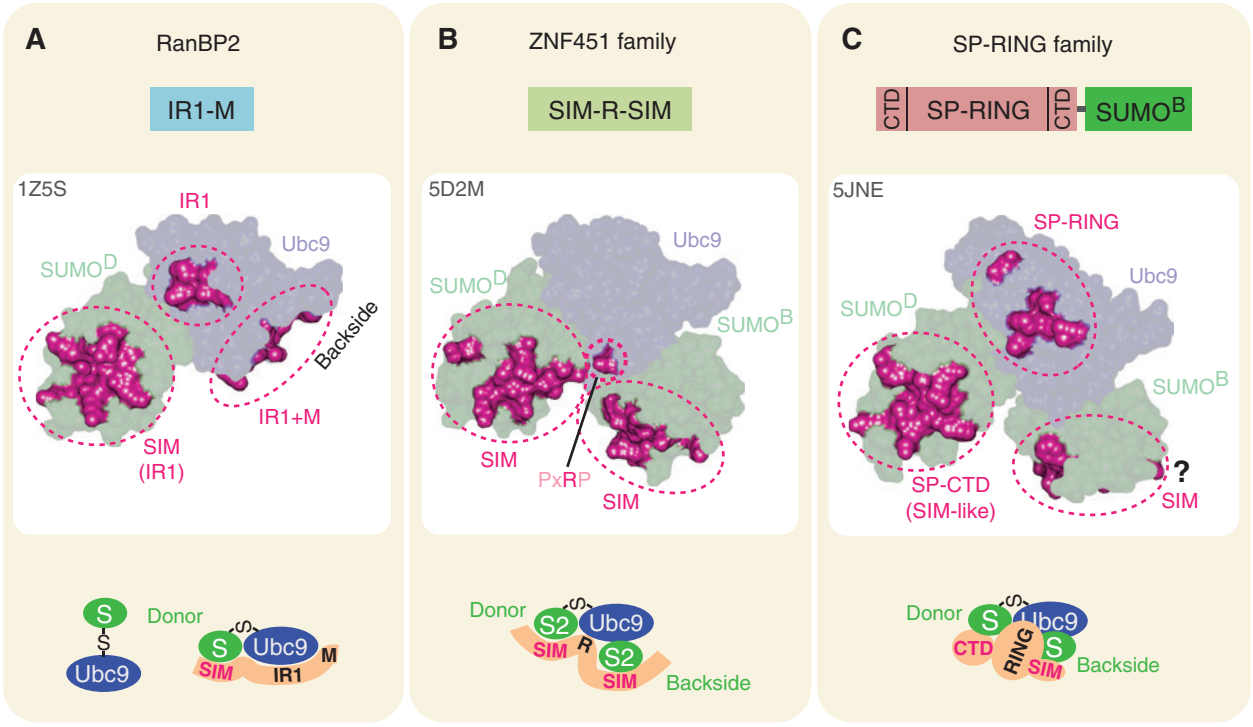


Figure 6: SUMO E3 catalysis.
Comparison of E3 binding interfaces with the SUMO^{D} charged Ubc9. Surface structures are shown without the E3 but highlighting the E3 interfaces (magenta) on the E2, SUMO^{D} and, where applicable, the scaffold SUMO^{B}. The simplified schematic cartoons below present these interactions in the presence of the respective minimal catalytic E3 domains used for crystallization and demonstrate how the individual E3s optimally orient SUMO^{D} in the closed conformation for the nucleophilic attack. In (A) the SUMO^{D}-loaded Ubc9 without an E3 is also shown, indicating a flexible SUMO^{D}. E3 binding interfaces on the surface structures are shown in magenta and cartoon models show Ubc9 in blue, SUMO in green and E3 in salmon. Thioester-bonds are indicated as -S-. (A) The minimal catalytic domain of RanBP2 is IR1+M, which wraps around Ubc9, interacting with the Ubc9 N-terminus and backside. A SIM (SUMO interaction motif) in the IR1 domain orients the donor SUMO^{D} in a closed conformation [1Z5S, (125)]. (B) The tandem SIM region of ZNF451 represents the minimal catalytic region for ZNF451 family members. It interacts with the charged E2 via two SIMs, one orients the donor SUMO in a closed conformation, while the second interacts with a scaffold SUMO in the backside of Ubc9. The interSIM PxRP motif wedges between the two SUMOs to allow the arginine to interact directly with Ubc9 [5DSM, (75)]. (C) For crystallization of the SP-RING E3 ligase Siz1 with the loaded Ubc9, a CTD-SP-RING-CTD-SUMO fusion was used. The SP-RING interacts with Ubc9, while the CTD domain positions the donor SUMO in a closed conformation via a SIM-like motif in the N-terminal CTD. Surprisingly, the fused SUMO^{B} stabilized the complex by binding to the backside of Ubc9 [5JNE, (71)]. All Pias family members possess a SIM C-terminal of the SP-RING that likely stabilizes the backside SUMO^{B} in the context of the full-length protein [cartoon, (87)].

the inter-SIM region, had dramatic consequences on the enzymatic activity, in agreement with the observation that multiple SIMs *per se* do not confer SUMO E3 ligase activity (75, 83). Concordantly, the tetraSIM region of RNF4 was unable to transfer SUMO although it becomes automodified at high enzyme concentrations (83). This is not surprising as, at least to some extent, SIM-containing proteins at high enzyme concentrations recruit the charged E2 enzyme, allowing cis- (auto-) but not trans- (substrate) modifications.

The latest and most complex crystal structure was solved for the SP-RING family member Siz1, interacting with a SUMO-charged E2 and its substrate, confirming and extending insights into SP-RING function (71). As proposed before by biochemical analyses, the SP-RING interacts with Ubc9 (115, 141, 142, 150) and the SUMO^{D} is positioned via the CTD domain flanking the SP-RING (150). The orientation of the SUMO^{D} was again found in an almost identical closed conformation to that seen for RanBP2 and ZNF451 (71). The crystal structure uncovered how SUMO interacts with the N-terminal part of the SP-CTD domain and revealed an alleviated unpredictable SIM [see above SUMO interactions, (71)]. Of note, MMS21 lacks an obvious N-terminal SP-CTD but contains hydrophobic residues in the corresponding region that might represent a SIM-like motif able to position the donor SUMO^{D}. Functional and/or structural analysis will be required to understand SUMO^{D} positioning for MMS21 proteins.

Functional studies indicated that a second SUMO^B in the backside of Ubc9 also promoted complex formation of E2~SUMO^D with E3-SUMO^B and its E3 ligase activity. Accordingly, a stabilizing fusion of a SUMO^B to the C-terminus of the Siz1 CTD-SP-RING-CTD fragment lacking the SIM was constructed for crystallization (71). This second SUMO^B interacted with the backside of Ubc9 in a way strongly resembling the scaffold SUMO^B interaction of ZNF451, although, in clear difference to ZNF451, the second SUMO^B merely stimulates Siz1 activity, while it is absolutely required for ZNF451 function. This key difference is likely due to the larger binding interface shared between Siz1 and Ubc9.

Further evidence for the significance of a scaffold SUMO^B interaction comes from Pias1, which interacts via its SIM, C-terminally flanking the SP-RING with a SUMO1^B on the backside of Ubc9 (87). Furthermore, Pias1 phosphorylation enhanced this ternary complex formation by engaging a phospho-SIM in the SUMO1^B interaction (87). Interestingly, earlier studies have shown that this phosphorylation influences the transcriptional coregulatory activities of Pias1 and other Pias family members (88), even though Pias1's SIM phosphorylation was proposed not to influence its catalytic activity. In light of these new findings, one would expect that stabilization of the active complex formed among SUMO^B, E2~SUMO^D and the phospho-E3 results in increased Pias1 E3 ligase activity, at least in a SUMO1 paralog-specific manner. Alternatively, a scaffold SUMO^B could also be provided by E3 autosumoylation (SP-RING and ZNF451 family members) that would stabilize the ternary complex. MMS21 and its yeast ortholog Nse2 do not possess a SIM C-terminal to the SP-RING as is conserved in all Siz/Pias proteins, but they could become automodified for ternary complex formation. It would be interesting to see whether automodification could provide a SUMO^B for the formation of a stable ternary complex or whether it only exists in a less stable dimeric complex.

In conclusion, all SUMO E3 ligases share a common, nearly identical feature of how they orient the donor SUMO^D in a closed conformation essential for their activities. A second scaffold SUMO^B interaction appears to play varying functions for individual E3 ligases. It is essential for the E3 activity of ZNF451 family members (83) and enhances the activity of Siz/Pias family members (71). However, MMS21 lacks the SIM flanking the SP-RING and RanBP2 directly interacts with the backside of Ubc9. In case of RanBP2, we cannot rule out a function for a scaffold SUMO^B in context of the larger IR1-M-IR2 (not in complex with sumoylated RanGAP1), as it also contains two SUMO binding sites. In general, it will be of great interest to gain further insights into the enigmatic functional role of SUMO-Ubc9 backside interactions and their role in E3 ligase function.

Substrate specificity

Per definition, E3 ligases provide specificity to the system by selecting the substrates. Currently, however, more than 6000 SUMO substrates in human cells (38) face 10 human E3 ligases. Thus, the extent to which SUMO E3 ligases execute substrate specificity remains one of the most enigmatic SUMO topics.

All SUMO E3 ligases seem to have a multiplicity of substrates but also appear to have unique targets. The global SUMO proteome is highly dynamic and constantly changes, for example, during the cell cycle or following a variety of different stress stimuli, resulting in stimulus-specific group sumoylation and desumoylation (13, 36, 38, 197). Analysis of all available proteomic datasets indicates a general switch from SCMs to non-SCM sumoylation upon stress (13). This raises the question whether E3 ligases 'promiscuously' modify substrates under these conditions, while constitutive SCM sumoylation is mainly E2-dependent. We could envision E2-dependent sumoylation to a certain extent, as discussed above, but what is known about E3 substrate specificity?

That E3 ligases indeed can dictate a sumoylation switch from a SCM to a non-SCM lysine is best documented for the proliferating cell nuclear antigen (PCNA) and its E3 ligase Siz1 (12, 71, 112). In the S-phase, sumoylation of the non-SCM Lys164 in PCNA is strictly dependent on Siz1, while SCM Lys127 modification is E3-independent [(12, 112); see also Figure 4]. Structural and biochemical analyses provided the first insights of how an E3 ligase dictates this lysine switch by showing that the interaction of PCNA with the Siz1-PINIT domain forces Lys164 into the E2 catalytic cleft (71). Mutational analysis in the E2 disclosed that the residues coordinating the non-SCM lysine modification were different from those used for the SCM lysine modification (55, 71). PCNA Lys164 sumoylation is highly enriched in the S-phase and facilitates the recruitment of the Srs2 helicase to prevent recombination in this phase of the cell cycle (12, 112). Consequently, this particular E3-directed lysine switch in PCNA appears to be very specific as it displays a precise mechanism of high biological importance.

As this is the first, and to our knowledge, the only example demonstrating how an E3 ligase determines the lysine choice in a substrate, it remains to be shown if this is a more general concept for E3-dependent sumoylation.

However, other examples of E3-dependent lysine changes were not detected, at least *in vitro* (124, 143, 192).

Several studies have shown (although often not published) that mutating the major sumoylation site(s) does not abolish sumoylation. The modification appears to jump to other lysines without any biological consequences (68, 198). Even modification of pathway-associated partner proteins is reported to be sufficient for executing sumoylation-dependent functions, as is discussed for the yeast septins (141) and several DNA repair factors (197). These findings are consistent with a study in *S. cerevisiae*, demonstrating that multiple domains in Siz1 and Siz2 contribute to substrate selection. However, although many substrates can be modified by either E3 ligase, other substrates are unique for the respective ligase (199). Interestingly, depletion of both Siz proteins strongly reduced, but did not completely abolish substrate sumoylation, and these substrates were only partially MMS21-dependent (199). This indicates that substrate sumoylation is often redundant between the individual E3 ligases and explains why E3 ligases are non-essential for yeast viability, in contrast to the E1 and the E2 enzymes. However, it also supports the idea of either the presence of undiscovered E3 ligases, or basal E2-dependent substrate modification that is sufficient to execute the minimal functions required for yeast viability.

Another important concept of how substrate modification can be regulated is by the spatial and temporal regulation that controls the co-occurrence of a subgroup of substrates with its E3 ligase, as was initially discovered for Siz1. Upon phosphorylation in mitosis, Siz1 is translocated to the bud neck where it meets its substrates, the septins (141). In general, different PTMs could regulate E3 localization, abundance, activity and substrate-specific interactions, indicating a tightly regulated system that only appears, at first glance, to exhibit promiscuous lysine or substrate selection.

How RanBP2 interacts with its substrate is still questioned. Initial studies could not demonstrate substrate interaction and biochemical analyses rather indicated allosteric activation of the charged Ubc9 (110, 124, 179, 193). *In vitro*, the RanBP2ΔFG and IR1-M-IR2 fragments showed some substrate specificity in comparison to the smaller IR1 or IR-M fragments, suggesting that regions flanking the minimal catalytic domain at least partially contribute to substrate selection (124, 200). Greater evidence that RanBP2ΔFG is indeed able to recognize substrates, even *in vivo*, was shown by the ectopic expression of RanBP2ΔFG, which is sufficient to bind and sumoylate its substrate Topo IIα in mitosis, restoring the RanBP2 depletion phenotype (187). An additional concept of how RanBP2 could recruit its substrates proposes that the flanking regions of its catalytic E3 ligase domain comprise several docking sites for nuclear transport receptors. It is appealing that such transport complexes are substrates for the multimeric RanBP2-S1*RanGAP1-Ubc9 complex (194, 201).

RanBP2 is highly sumoylated *in vivo* and *in vitro*, with more than 20 lysines close to the catalytic domain being modified (13). Such automodifications could also serve to recruit selected SIM-containing substrates, for example, the model substrate Sp100.

For ZNF451, two substrate-binding interfaces have been described. One is the zinc finger region that bears a functionally uncharacterized SUMO-binding interface required for SUMO chain initiation (83). The same zinc finger region is also required for PML sumoylation *in vitro*, suggesting that it acts as a platform for different substrate interactions. This particular region is specific to ZNF451-1 and ZNF451-2 but absent in ZNF451-3 and KIAA1586, and in agreement, ZNF451-3 is inefficient in PML sumoylation compared to ZNF451-1 (192). The minichromosome-maintenance-4-protein (MCM4), a subunit of the replication fork helicase, was identified as the first ZNF451 *in vivo* SUMO substrate (83), although *in vitro* sumoylation and mapping of the binding region remains to be shown. A clearly distinct substrate interface is provided by the second SIM that binds the SUMO in the backside of Ubc9 (83). This interface is of particular interest because a SUMO chain (but not a single SUMO) anchored in this position can be efficiently extended by the ZNF451 tandem-SIM region. This revealed the scaffold SUMO position as a substrate-binding interface specified for SUMO chain extension and disclosed an E4 elongase function for the ZNF451 family members.

In general, substrate selection and specificity of SUMO E3 ligases is a still poorly resolved and an important topic to be addressed in future research. From the currently available data, it appears that E3 ligase-specific interactions, combined with the timely and spatial co-existence of substrate and enzymes, regulate substrate choice.

SUMO chains

In the ubiquitin system, different chain linkages create a variety of signals that determine the fate of the modified protein (202). By contrast, SUMO chains are mainly assembled on SUMO consensus site lysines in the flexible N-terminus unique to SUMO proteins (Lys11 in SUMO2/3 and Lys11, 15 and 19 in SMT3). SUMO1 bears an inverted

SCM (ExK) involving Lys7 and chain assembly via this site is the most prominent linkage identified *in vivo* (38). Currently, there is no indication that different SUMO linkages result in different signals. To date, SUMO chains are best understood as tags that recruit SUMO-targeted ubiquitin E3 ligases (StUbls) like RNF4 and RNF111/Arkadia, which subsequently mark sumo(chain)ylated proteins with K48-linked ubiquitin chains for their proteasomal degradation (64, 67). Alternatively, StUbls can attach K63-linked ubiquitin chains as a signal important for the DNA damage response (203). While SUMO chains are required for the recruitment of SIM-containing proteins to DNA lesions (117, 203, 204), it is currently unclear if such chains are free unanchored or substrate-linked SUMO chains. In *S. cerevisiae*, SUMO chains are implicated to have important roles for synaptonemal complex formation in meiosis (68, 205), in the organization of higher-order chromatin and the transcriptional repression of environmental stress-response genes (206).

SUMO chain formation is a common feature of all SUMO enzymes. The E1 and the E2 enzymes, by themselves, can assemble SUMO chains *in vitro*, although higher concentrations are required than in E3 ligase-dependent reactions. Mechanistic insights into how chains are assembled are still limited, but the molecular analysis of E2 and E3 enzymes has revealed unexpected clues. Biochemical studies of C-terminal Ubc9 sumoylation at Lys153, as it is found in *S. cerevisiae*, indicated that this particular modification inactivated Ubc9 in its classical E2 functions but turned it into a co-factor for SUMO chain assembly. Mechanistically, the sumoylated E2 recruits a charged unmodified E2 via its backside and positions the donor SUMO^{D} in its catalytic cleft [Figure 7, left panel; (68)]. However, the sumoylated E2-dependent enhancement of chain formation is rather poor, most likely because the substrate (acceptor) SUMO is not stabilized in the complex. Of note, this mechanism is yeast-specific as the mammalian Ubc9 is sumoylated at its N-terminus (69).

Novel insights into SUMO chain assembly came from the functional analysis of the tandem-SIM region of ZNF451. This region can extend a short SUMO chain from the backside of Ubc9, while chain initiation requires the adjacent zinc finger region [Figure 7, right panel; (83)]. SUMO chain extension from Ubc9's backside could be a more general mechanism as in *S. cerevisiae*, the sumoylated E2 possesses a free backside that could anchor a SUMO chain. In line with this, a similar mechanism may apply for Siz/Pias E3 ligases as a backside SUMO^{B} enhances their E3 ligase activity. Indeed, an E2 mutant impaired in SUMO backside interaction displays significantly reduced Pias1-dependent SUMO2 chain formation activity (N.E. and A.P., unpublished results). MMS21 and Nse2 lack this SIM C-terminal to the SP-RING and, therefore, it would be of great interest to

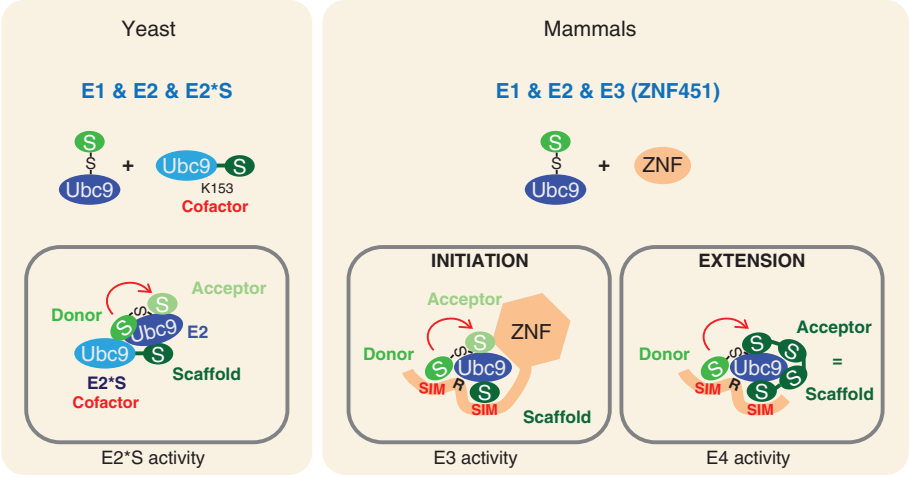


Figure 7: SUMO chain assembly.
Two mechanisms of SUMO chain assembly are described. Left panel: SUMO chain formation in *S. cerevisiae* by a charged Ubc9 and a sumoylated Ubc9. C-terminal Ubc9 sumoylation on Lys153 (Ubc9 in light blue and SUMO in dark green) in *S. cerevisiae* inactivates Ubc9 in its classical E2 function but turns it into a cofactor for SUMO chain formation. It binds to the backside of a SUMO^{D}-charged Ubc9 (SUMO^{D} in middle green and Ubc9 in dark blue) and positions the donor SUMO^{D} in its catalytic cleft. The acceptor SUMO (light green) binds to the catalytic cleft of the loaded Ubc9 (68). Right panel: SUMO chain formation by SUMO^{D}-charged Ubc9 and ZNF451 in mammals. The tandem SIM region of ZNF451 family members (salmon) is sufficient to extend a short SUMO chain (dark green) anchored in the scaffold SUMO^{B} position on the backside of Ubc9. Chain initiation requires an additional binding interface, the ZNF-region, to recruit the first acceptor SUMO (light green) (83).

compare its chain-forming ability with the Pias family members in greater detail.

How RanBP2 assembles SUMO chains is still in question because it directly interacts with the backside of Ubc9. It is likely that cooperation between both internal repeats is required for SUMO chain assembly. As both internal repeats are only accessible in the absence of sumoylated RanGAP1, as is the case in differentiated cells (196), it would be interesting to investigate RanBP2's chain-forming ability in the absence and presence of sumoylated RanGAP1 *in vitro* and in undifferentiated vs. differentiated cells.

Expert opinion

Since its discovery 20 years ago, our mechanistic understanding of protein sumoylation has greatly expanded. Often seen as just the 'small brother' of ubiquitin, many similarities have been found, although over time, several unique features have become evident (SCM, diverse E2-substrate interactions, high-affinity E2-SUMO backside interaction, chain formation). One unresolved key question is how sumoylation achieves substrate specificity with its limited number of enzymes, compared to the large number of substrates. All identified E3 ligases appear to have both unique and promiscuous substrates. This raises the question as to whether the spatial and temporal colocalization of modifying and demodifying enzymes is sufficient or whether other mechanisms contribute to achieve specificity. Or does sumoylation work as spray paint upon stress induction, modifiying everything in close proximity to its E2 and E3 enzymes? One can find examples supporting both models, like the highly regulated modification of PCNA or the septins compared to the seemingly unspecific group sumoylation upon stress. As global changes in the SUMO proteome depend on the particular stimulus, it is likely that they represent tightly regulated subgroups of proteins that are specific for the respective treatment. The newly improved techniques in mass spectrometry, in combination with cellular, biochemical and structural analyses, should provide exciting new insights into the mechanism of SUMO specificity.

Recent functional and structural studies have revealed a common mechanism for SUMO E3 ligase catalysis, highlighting the importance of Ubc9's backside and the requirement of SUMO interactions to orient SUMO^{D} in a highly reactive closed conformation (71, 75, 83, 124, 125). This closed conformation, initially identified for the SUMO E3 ligase RanBP2 (125), seems to represent a unifying feature of the majority of E3 ligases in the Ubl system, as it also is seen in ubiquitin RING and U-box E3 ligases (207, 208). The importance of the Ubc9-SUMO^{B} backside interaction seems to be specific for the SUMO system, where this particular interaction shows a 1000 times higher affinity than its counterparts in the ubiquitin system. However, a constitutive SUMO^{B} on the backside of Ubc9 is unlikely to form because this surface partially overlaps with the E1 interaction and needs to be displaced for every new round of E2 charging (78).

Outlook

The biological requirements and consequences of dynamic global changes in the SUMO proteome and the underlying mechanistic insights into substrate specificity of the SUMO system are major open questions. We can envision the existence of additional E3 ligases and expect more regulatory concepts for E2, E3 and demodifying enzymes that define the equilibrium for substrate modification. Currently, we are just at the beginning of decoding enzyme-substrate interactions and we do not yet understand whether increased E2-substrate affinities are sufficient for *in vivo* substrate sumoylation. Also, the importance of site-specific substrate modification is heavily discussed, as for some substrates, the exact position is crucial, while for others it seems not to matter as long as they are modified. In selected cases, it even appears sufficient to modify one or the other partner protein in a multi-protein complex. Unraveling the substrate spectra for each E3 ligase and the E2 regulatory mechanism evoked by different stimuli, combined with the fine mapping of substrate-enzyme interactions and their structural analysis, will be of cardinal importance to understand the specificity of the SUMO system and the functional importance of sumoylation.

Highlights

- Sumoylation is a rapid and versatile tag which changes protein function.
- Sumoylation is highly dynamic through the opposing activities of SUMO conjugating (E1, E2 and E3) and deconjugating (proteases) enzymes.
- Covalent and non-covalent SUMO interactions mediate SUMO dependent functions.
- Sumoylation often targets lysines embedded in a SCM (ΨKxE); non-consensus lysines are also sumoylated.

- SUMO E2 and E3 enzymes can select substrates for sumoylation.
- E2-dependent sumoylation is mainly executed by increased binding affinities between the charged E2 and the substrate.
- E3 enzymes interact with the charged E2 and the substrate to bring them in close proximity. In addition, these enzymes orient the donor SUMO ($SUMO^D$) in a highly reactive closed conformation to accelerate the $SUMO^D$ transfer.
- Substrate selection and specificity is still poorly understood.

Acknowledgments: We apologize to all colleagues whose work could not be cited owing to space limitations. We thank Dr. P. Nielsen for critical reading of the manuscript. The Pichler laboratory is funded by the Max Planck Society and the Wilhelm Sanders Stiftung.

Conflict of interest statement: The authors declare no conflict of financial or other interests.

List of abbreviations

PTM	posttranslational modification
SUMO	small ubiquitin-related modifier
Ubl	ubiquitin-like
SIM	SUMO interaction motif
SCM	SUMO consensus motif
HCSM	hydrophobic cluster sumoylation motif
NDSM	negatively charged amino-acid-dependent sumoylation motif
PDSM	phosphorylation-dependent sumoylation motif
Ubc	ubiquitin conjugation
SLD	SUMO-like domain
SUMOD	donor SUMO (charged on Ubc9)
SUMOB	backside-bound SUMO (bound by the backside of Ubc9)
UIM	ubiquitin interaction motif
Ubc9	ubiquitin conjugating enzyme
StUbls	SUMO-targeted ubiquitin ligase

References

1. Droescher M, Chaugule VK, Pichler A. SUMO rules: regulatory concepts and their implication in neurologic functions. Neuromol Med 2013; 15: 639–60.
2. Nie M, Boddy MN. Cooperativity of the SUMO and Ubiquitin Pathways in Genome Stability. Biomolecules 2016; 6: 14.
3. Chymkowitch P, Nguea PA, Enserink JM. SUMO-regulated transcription: challenging the dogma. Bioessays 2015; 37: 1095–105.
4. Flotho A, Melchior F. Sumoylation: a regulatory protein modification in health and disease. Annu Rev Biochem 2013; 82: 357–85.
5. Cubenas-Potts C, Matunis MJ. SUMO: a multifaceted modifier of chromatin structure and function. Dev Cell 2013; 24: 1–12.
6. Geoffroy MC, Hay RT. An additional role for SUMO in ubiquitin-mediated proteolysis. Nat Rev Mol Cell Biol 2009; 10: 564–8.
7. Garcia-Rodriguez N, Wong RP, Ulrich HD. Functions of ubiquitin and SUMO in DNA replication and replication stress. Front Genet 2016; 7: 87.
8. Eifler K, Vertegaal AC. SUMOylation-mediated regulation of cell cycle progression and cancer. Trends Biochem Sci 2015; 40: 779–93.
9. Mattoscio D, Segre CV, Chiocca S. Viral manipulation of cellular protein conjugation pathways: the SUMO lesson. World J Virol 2013; 2: 79–90.
10. Guo B, Yang SH, Witty J, Sharrocks AD. Signalling pathways and the regulation of SUMO modification. Biochem Soc Trans 2007; 35(Pt 6): 1414–8.
11. Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature 2002; 419: 135–41.
12. Pfander B, Moldovan GL, Sacher M, Hoege C, Jentsch S. SUMO-modified PCNA recruits Srs2 to prevent recombination during S phase. Nature 2005; 436: 428–33.
13. Hendriks IA, Vertegaal AC. A comprehensive compilation of SUMO proteomics. Nat Rev Mol Cell Biol 2016; 17: 581–95.
14. Cubenas-Potts C, Srikumar T, Lee C, Osula O, Subramonian D, Zhang XD, Cotter RJ, Raught B, Matunis MJ. Identification of SUMO-2/3-modified proteins associated with mitotic chromosomes. Proteomics 2015; 15: 763–72.
15. Hendriks IA, D'Souza RC, Yang B, Verlaan-de Vries M, Mann M, Vertegaal AC. Uncovering global SUMOylation signaling networks in a site-specific manner. Nat Struct Mol Biol 2014; 21: 927–36.
16. Henley JM, Craig TJ, Wilkinson KA. Neuronal SUMOylation: mechanisms, physiology, and roles in neuronal dysfunction. Physiol Rev 2014; 94: 1249–85.
17. Schimmel J, Eifler K, Sigurethsson JO, Cuijpers SA, Hendriks IA, Verlaan-de Vries M, Kelstrup CD, Francavilla C, Medema RH, Olsen JV. Uncovering SUMOylation dynamics during cell-cycle progression reveals FoxM1 as a key mitotic SUMO target protein. Mol Cell 2014; 53: 1053–66.
18. Dantuma NP, Pfeiffer A. Real estate in the DNA damage response: ubiquitin and SUMO ligases home in on DNA double-strand breaks. Front Genet 2016; 7: 58.
19. Lee YJ, Mou Y, Maric D, Klimanis D, Auh S, Hallenbeck JM. Elevated global SUMOylation in Ubc9 transgenic mice protects their brains against focal cerebral ischemic damage. PLoS One 2011; 6: e25852.
20. Chen SF, Gong C, Luo M, Yao HR, Zeng YJ, Su FX. Ubc9 expression predicts chemoresistance in breast cancer. Chin J Cancer 2011; 30: 638–44.
21. Du L, Li YJ, Fakih M, Wiatrek RL, Duldulao M, Chen Z, Chu P, Garcia-Aguilar J, Chen Y. Role of SUMO activating enzyme in cancer stem cell maintenance and self-renewal. Nat Commun 2016; 7: 12326.
22. Moschos SJ, Mo YY. Role of SUMO/Ubc9 in DNA damage repair and tumorigenesis. J Mol Histol 2006; 37: 309–19.
23. Qin Y, Bao H, Pan Y, Yin M, Liu Y, Wu S, Li H. SUMOylation alterations are associated with multidrug resistance in hepatocellular carcinoma. Mol Med Rep 2014; 9: 877–81.

24. Zhu S, Sachdeva M, Wu F, Lu Z, Mo YY. Ubc9 promotes breast cell invasion and metastasis in a sumoylation-independent manner. Oncogene 2010; 29: 1763–72.
25. Eckermann K. SUMO and Parkinson's disease. Neuromolecular Med 2013; 15: 737–59.
26. Martins WC, Tasca CI, Cimarosti H. Battling alzheimer's disease: targeting SUMOylation-mediated pathways. Neurochem Res 2016; 41: 568–78.
27. Mendler L, Braun T, Muller S. The ubiquitin-like SUMO system and heart function: from development to disease. Circ Res 2016; 118: 132–44.
28. Sireesh D, Bhakkiyalakshmi E, Ramkumar KM, Rathinakumar S, Jennifer PS, Rajaguru P, Paulmurugan R. Targeting SUMOylation cascade for diabetes management. Curr Drug Targets 2014; 15: 1094–106.
29. Hickey CM, Wilson NR, Hochstrasser M. Function and regulation of SUMO proteases. Nat Rev Mol Cell Biol 2012; 13: 755–66.
30. Nayak A, Muller S. SUMO-specific proteases/isopeptidases: SENPs and beyond. Genome Biol 2014; 15: 422.
31. Schulman BA, Harper JW. Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways. Nat Rev Mol Cell Biol 2009; 10: 319–31.
32. Lois LM, Lima CD. Structures of the SUMO E1 provide mechanistic insights into SUMO activation and E2 recruitment to E1. EMBO J 2005; 24: 439–51.
33. Wang J, Taherbhoy AM, Hunt HW, Seyedin SN, Miller DW, Miller DJ, Huang DT, Schulman BA. Crystal structure of UBA2(ufd)-Ubc9: insights into E1-E2 interactions in Sumo pathways. PLoS One 2010; 5: e15805.
34. Mahajan R, Gerace L, Melchior F. Molecular characterization of the SUMO-1 modification of RanGAP1 and its role in nuclear envelope association. J Cell Biol 1998; 140: 259–70.
35. Matunis MJ, Coutavas E, Blobel G. A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. J Cell Biol 1996; 135: 1457–70.
36. Johnson ES, Blobel G. Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. J Biol Chem 1997; 272: 26799–802.
37. Bayer P, Arndt A, Metzger S, Mahajan R, Melchior F, Jaenicke R, Becker J. Structure determination of the small ubiquitin-related modifier SUMO-1. J Mol Biol 1998; 280: 275–86.
38. Hendriks IA, Lyon D, Young C, Jensen LJ, Vertegaal AC, Nielsen ML. Site-specific mapping of the human SUMO proteome reveals co-modification with phosphorylation. Nat Struct Mol Biol 2017. doi: 10.1038/nsmb.3366.
39. Evdokimov E, Sharma P, Lockett SJ, Lualdi M, Kuehn MR. Loss of SUMO1 in mice affects RanGAP1 localization and formation of PML nuclear bodies, but is not lethal as it can be compensated by SUMO2 or SUMO3. J Cell Sci 2008; 121(Pt 24): 4106–13.
40. Zhang FP, Mikkonen L, Toppari J, Palvimo JJ, Thesleff I, Janne OA. Sumo-1 function is dispensable in normal mouse development. Mol Cell Biol 2008; 28: 5381–90.
41. Wang L, Wansleeben C, Zhao S, Miao P, Paschen W, Yang W. SUMO2 is essential while SUMO3 is dispensable for mouse embryonic development. EMBO Rep 2014; 15: 878–85.
42. Saitoh H, Hinchey J. Functional heterogeneity of small ubiquitin-related protein modifiers SUMO-1 versus SUMO-2/3. J Biol Chem 2000; 275: 6252–8.
43. Bohren KM, Nadkarni V, Song JH, Gabbay KH, Owerbach D. A M55V polymorphism in a novel SUMO gene (SUMO-4) differentially activates heat shock transcription factors and is associated with susceptibility to type I diabetes mellitus. J Biol Chem 2004; 279: 27233–8.
44. Guo D, Li M, Zhang Y, Yang P, Eckenrode S, Hopkins D, Zheng W, Purohit S, Podolsky RH, Muir A, Wang J, Dong Z, Brusko T, Atkinson M, Pozzilli P, Zeidler A, Raffel LJ, Jacob CO, Park Y, Serrano-Rios M, Larrad MT, Zhang Z, Garchon HJ, Bach JF, Rotter JI, She JX, Wang CY. A functional variant of SUMO4, a new I kappa B alpha modifier, is associated with type 1 diabetes. Nat Genet 2004; 36: 837–41.
45. Liang YC, Lee CC, Yao YL, Lai CC, Schmitz ML, Yang WM. SUMO5, a Novel Poly-SUMO Isoform, Regulates PML Nuclear Bodies. Sci Rep 2016; 6: 26509.
46. Su HL, Li SS. Molecular features of human ubiquitin-like SUMO genes and their encoded proteins. Gene 2002; 296: 65–73.
47. Tatham MH, Jaffray E, Vaughan OA, Desterro JM, Botting CH, Naismith JH, Hay RT. Polymeric chains of SUMO-2 and SUMO-3 are conjugated to protein substrates by SAE1/SAE2 and Ubc9. J Biol Chem 2001; 276: 35368–74.
48. Wei W, Yang P, Pang J, Zhang S, Wang Y, Wang MH, Dong Z, She JX, Wang CY. A stress-dependent SUMO4 sumoylation of its substrate proteins. Biochem Biophys Res Commun 2008; 375: 454–9.
49. Ullmann R, Chien CD, Avantaggiati ML, Muller S. An acetylation switch regulates SUMO-dependent protein interaction networks. Mol Cell 2012; 46: 759–70.
50. Lin CH, Liu SY, Lee EH. SUMO modification of Akt regulates global SUMOylation and substrate SUMOylation specificity through Akt phosphorylation of Ubc9 and SUMO1. Oncogene 2016; 35: 595–607.
51. Bursomanno S, Beli P, Khan AM, Minocherhomji S, Wagner SA, Bekker-Jensen S, Mailand N, Choudhary C, Hickson ID, Liu Y. Proteome-wide analysis of SUMO2 targets in response to pathological DNA replication stress in human cells. DNA Repair (Amst) 2015; 25: 84–96.
52. Impens F, Radoshevich L, Cossart P, Ribet D. Mapping of SUMO sites and analysis of SUMOylation changes induced by external stimuli. Proc Natl Acad Sci USA 2014; 111: 12432–7.
53. Lamoliatte F, Caron D, Durette C, Mahrouche L, Maroui MA, Caron-Lizotte O, Bonneil E, Chelbi-Alix MK, Thibault P. Large-scale analysis of lysine SUMOylation by SUMO remnant immunoaffinity profiling. Nat Commun 2014; 5: 5409.
54. Tammsalu T, Matic I, Jaffray EG, Ibrahim AF, Tatham MH, Hay RT. Proteome-wide identification of SUMO2 modification sites. Sci Signal 2014; 7: rs2.
55. Bernier-Villamor V, Sampson DA, Matunis MJ, Lima CD. Structural basis for E2-mediated SUMO conjugation revealed by a complex between ubiquitin-conjugating enzyme Ubc9 and RanGAP1. Cell 2002; 108: 345–56.
56. Yunus AA, Lima CD. Lysine activation and functional analysis of E2-mediated conjugation in the SUMO pathway. Nat Struct Mol Biol 2006; 13: 491–9.
57. Matic I, Schimmel J, Hendriks IA, van Santen MA, van de Rijke F, van Dam H, Gnad F, Mann M, Vertegaal AC. Site-specific identification of SUMO-2 targets in cells reveals an inverted SUMOylation motif and a hydrophobic cluster SUMOylation motif. Mol Cell 2010; 39: 641–52.

58. Yang SH, Galanis A, Witty J, Sharrocks AD. An extended consensus motif enhances the specificity of substrate modification by SUMO. EMBO J 2006; 25: 5083–93.
59. Hietakangas V, Anckar J, Blomster HA, Fujimoto M, Palvimo JJ, Nakai A, Sistonen L. PDSM, a motif for phosphorylation-dependent SUMO modification. Proc Natl Acad Sci USA 2006; 103: 45–50.
60. Mohideen F, Capili AD, Bilimoria PM, Yamada T, Bonni A, Lima CD. A molecular basis for phosphorylation-dependent SUMO conjugation by the E2 UBC9. Nat Struct Mol Biol 2009; 16: 945–52.
61. Picard N, Caron V, Bilodeau S, Sanchez M, Mascle X, Aubry M, Tremblay A. Identification of estrogen receptor beta as a SUMO-1 target reveals a novel phosphorylated sumoylation motif and regulation by glycogen synthase kinase 3b. Mol Cell Biol 2012; 32: 2709–21.
62. Pichler A, Knipscheer P, Oberhofer E, van Dijk WJ, Korner R, Olsen JV, Jentsch S, Melchior F, SixmaTK. SUMO modification of the ubiquitin-conjugating enzyme E2-25K. Nat Struct Mol Biol 2005; 12: 264–9.
63. Desterro JM, Rodriguez MS, Hay RT. SUMO-1 modification of IkBa inhibits NF-kB activation. Mol Cell 1998; 2: 233–9.
64. Lallemand-Breitenbach V, Jeanne M, Benhenda S, Nasr R, Lei M, Peres L, Zhou J, Zhu J, Raught B, de Thé H. Arsenic degrades PML or PML-RARalpha through a SUMO-triggered RNF4/ubiquitin-mediated pathway. Nat Cell Biol 2008; 10: 547–55.
65. Mahajan R, Delphin C, Guan T, Gerace L, Melchior F. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. Cell 1997; 88: 97–107.
66. Matunis MJ, Wu J, Blobel G. SUMO-1 modification and its role in targeting the Ran GTPase-activating protein, RanGAP1, to the nuclear pore complex. J Cell Biol 1998; 140: 499–509.
67. Tatham MH, Geoffroy MC, Shen L, Plechanovova A, Hattersley N, Jaffray EG, Palvimo JJ, Hay RT. RNF4 is a poly-SUMO-specific E3 ubiquitin ligase required for arsenic-induced PML degradation. Nat Cell Biol 2008; 10: 538–46.
68. Klug H, Xaver M, Chaugule VK, Koidl S, Mittler G, Klein F, Pichler A. Ubc9 sumoylation controls SUMO chain formation and meiotic synapsis in Saccharomyces cerevisiae. Mol Cell 2013; 50: 625–36.
69. Knipscheer P, Flotho A, Klug H, Olsen JV, van Dijk WJ, Fish A, Johnson ES, Mann M, Sixma TK, Pichler A. Ubc9 sumoylation regulates SUMO target discrimination. Mol Cell 2008; 31: 371–82.
70. Praefcke GJ, Hofmann K, Dohmen RJ. SUMO playing tag with ubiquitin. Trends Biochem Sci 2012; 37: 23–31.
71. Streich FC, Jr., Lima CD. Capturing a substrate in an activated RING E3/E2-SUMO complex. Nature 2016; 536: 304–8.
72. Knipscheer P, van Dijk WJ, Olsen JV, Mann M, Sixma TK. Noncovalent interaction between Ubc9 and SUMO promotes SUMO chain formation. EMBO J 2007; 26: 2797–807.
73. Song J, Durrin LK, Wilkinson TA, Krontiris TG, Chen Y. Identification of a SUMO-binding motif that recognizes SUMO-modified proteins. Proc Natl Acad Sci USA 2004; 101: 14373–8.
74. Cappadocia L, Mascle XH, Bourdeau V, Tremblay-Belzile S, Chaker-Margot M, Lussier-Price M, Wada J, Sakaguchi K, Aubry M, Ferbeyre G, Omichinski JG. Structural and functional characterization of the phosphorylation-dependent interaction between PML and SUMO1. Structure 2015; 23: 126–38.
75. Cappadocia L, Pichler A, Lima CD. Structural basis for catalytic activation by the human ZNF451 SUMO E3 ligase. Nat Struct Mol Biol 2015; 22: 968–75.
76. Chang CC, Naik MT, Huang YS, Jeng JC, Liao PH, Kuo HY, Ho CC, Hsieh YL, Lin CH, Huang NJ, Naik NM, Kung CC, Lin SY, Chen RH, Chang KS, Huang TH, Shih HM. Structural and functional roles of Daxx SIM phosphorylation in SUMO paralog-selective binding and apoptosis modulation. Mol Cell 2011; 42: 62–74.
77. Capili AD, Lima CD. Structure and analysis of a complex between SUMO and Ubc9 illustrates features of a conserved E2-Ubl interaction. J Mol Biol 2007; 369: 608–18.
78. Duda DM, van Waardenburg RC, Borg LA, McGarity S, Nourse A, Waddell MB, Bjornsti MA, Schulman BA. Structure of a SUMO-binding-motif mimic bound to Smt3p-Ubc9p: conservation of a non-covalent ubiquitin-like protein-E2 complex as a platform for selective interactions within a SUMO pathway. J Mol Biol 2007; 369: 619–30.
79. Liu Q, Jin C, Liao X, Shen Z, Chen DJ, Chen Y. The binding interface between an E2 (UBC9) and a ubiquitin homologue (UBL1). J Biol Chem 1999; 274: 16979–87.
80. Pilla E, Moller U, Sauer G, Mattiroli F, Melchior F, Geiss-Friedlander R. A novel SUMO1-specific interacting motif in dipeptidyl peptidase 9 (DPP9) that is important for enzymatic regulation. J Biol Chem 2012; 287: 4420–9.
81. Danielsen JR, Povlsen LK, Villumsen BH, Streicher W, Nilsson J, Wikstrom M, Bekker-Jensen S, Mailand N. DNA damage-inducible SUMOylation of HERC2 promotes RNF8 binding via a novel SUMO-binding Zinc finger. J Cell Biol 2012; 197: 179–87.
82. Diehl C, Akke M, Bekker-Jensen S, Mailand N, Streicher W, Wikstrom M. Structural Analysis of a Complex between Small Ubiquitin-like Modifier 1 (SUMO1) and the ZZ Domain of CREB-binding Protein (CBP/p300) Reveals a New Interaction Surface on SUMO. J Biol Chem 2016; 291: 12658–72.
83. Eisenhardt N, Chaugule VK, Koidl S, Droescher M, Dogan E, Rettich J, Sutinen P, Imanishi SY, Hofmann K, Palvimo JJ, Pichler A. A new vertebrate SUMO enzyme family reveals insights into SUMO-chain assembly. Nat Struct Mol Biol 2015; 22: 959–67.
84. Meulmeester E, Kunze M, Hsiao HH, Urlaub H, Melchior F. Mechanism and consequences for paralog-specific sumoylation of ubiquitin-specific protease 25. Mol Cell 2008; 30: 610–9.
85. Ouyang J, Shi Y, Valin A, Xuan Y, Gill G. Direct binding of CoREST1 to SUMO-2/3 contributes to gene-specific repression by the LSD1/CoREST1/HDAC complex. Mol Cell 2009; 34: 145–54.
86. Anamika, Spyracopoulos L. Molecular basis for phosphorylation-dependent SUMO recognition by the DNA repair protein RAP80. J Biol Chem 2016; 291: 4417–28.
87. Mascle XH, Lussier-Price M, Cappadocia L, Estephan P, Raiola L, Omichinski JG, Aubry M. Identification of a non-covalent ternary complex formed by PIAS1, SUMO1, and UBC9 proteins involved in transcriptional regulation. J Biol Chem 2013; 288: 36312–27.
88. Stehmeier P, Muller S. Phospho-regulated SUMO interaction modules connect the SUMO system to CK2 signaling. Mol Cell 2009; 33: 400–9.
89. Hecker CM, Rabiller M, Haglund K, Bayer P, Dikic I. Specification of SUMO1- and SUMO2-interacting motifs. J Biol Chem 2006; 281: 16117–27.
90. Olsen SK, Capili AD, Lu X, Tan DS, Lima CD. Active site remodelling accompanies thioester bond formation in the SUMO E1. Nature 2010; 463: 906–12.

91. Desterro JM, Rodriguez MS, Kemp GD, Hay RT. Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1. J Biol Chem 1999; 274: 10618–24.
92. Johnson ES, Schwienhorst I, Dohmen RJ, Blobel G. The ubiquitin-like protein Smt3p is activated for conjugation to other proteins by an Aos1p/Uba2p heterodimer. EMBO J 1997; 16: 5509–19.
93. Dohmen RJ, Stappen R, McGrath JP, Forrova H, Kolarov J, Goffeau A, Varshavsky A. An essential yeast gene encoding a homolog of ubiquitin-activating enzyme. J Biol Chem 1995; 270: 18099–109.
94. Bossis G, Melchior F. Regulation of SUMOylation by reversible oxidation of SUMO conjugating enzymes. Mol Cell 2006; 21: 349–57.
95. Bossis G, Sarry JE, Kifagi C, Ristic M, Saland E, Vergez F, Salem T, Boutzen H, Baik H, Brockly F, Pelegrin M, Kaoma T, Vallar L, Récher C, Manenti S, Piechaczyk M. The ROS/SUMO axis contributes to the response of acute myeloid leukemia cells to chemotherapeutic drugs. Cell Rep 2014; 7: 1815–23.
96. Truong K, Lee TD, Chen Y. Small ubiquitin-like modifier (SUMO) modification of E1 Cys domain inhibits E1 Cys domain enzymatic activity. J Biol Chem 2012; 287: 15154–63.
97. Boggio R, Colombo R, Hay RT, Draetta GF, Chiocca S. A mechanism for inhibiting the SUMO pathway. Mol Cell 2004; 16: 549–61.
98. He X, Riceberg J, Pulukuri SM, Grossman S, Shinde V, Shah P, Brownell JE, Dick L, Newcomb J, Bence N. Characterization of the loss of SUMO pathway function on cancer cells and tumor proliferation. PLoS One 2015; 10: e0123882.
99. Amelio I, Landre V, Knight RA, Lisitsa A, Melino G, Antonov AV. Polypharmacology of small molecules targeting the ubiquitin-proteasome and ubiquitin-like systems. Oncotarget 2015; 6: 9646–56.
100. Hoeller D, Dikic I. Targeting the ubiquitin system in cancer therapy. Nature 2009; 458: 438–44.
101. Tong H, Hateboer G, Perrakis A, Bernards R, Sixma TK. Crystal structure of murine/human Ubc9 provides insight into the variability of the ubiquitin-conjugating system. J Biol Chem 1997; 272: 21381–7.
102. Matuschewski K, Hauser HP, Treier M, Jentsch S. Identification of a novel family of ubiquitin-conjugating enzymes with distinct amino-terminal extensions. J Biol Chem 1996; 271: 2789–94.
103. Olsen SK, Lima CD. Structure of a ubiquitin E1-E2 complex: insights to E1-E2 thioester transfer. Mol Cell 2013; 49: 884–96.
104. Nacerddine K, Lehembre F, Bhaumik M, Artus J, Cohen-Tannoudji M, Babinet C, Pandolfi PP, Dejean A. The SUMO pathway is essential for nuclear integrity and chromosome segregation in mice. Dev Cell 2005; 9: 769–79.
105. Hayashi T, Seki M, Maeda D, Wang W, Kawabe Y, Seki T, Saitoh H, Fukagawa T, Yagi H, Enomoto T. Ubc9 is essential for viability of higher eukaryotic cells. Exp Cell Res 2002; 280: 212–21.
106. Nowak M, Hammerschmidt M. Ubc9 regulates mitosis and cell survival during zebrafish development. Mol Biol Cell 2006; 17: 5324–36.
107. Seufert W, Futcher B, Jentsch S. Role of a ubiquitin-conjugating enzyme in degradation of S- and M-phase cyclins. Nature 1995; 373: 78–81.
108. al-Khodairy F, Enoch T, Hagan IM, Carr AM. The Schizosaccharomyces pombe hus5 gene encodes a ubiquitin conjugating enzyme required for normal mitosis. J Cell Sci 1995; 108 (Pt 2): 475–86.
109. Schwarz SE, Matuschewski K, Liakopoulos D, Scheffner M, Jentsch S. The ubiquitin-like proteins SMT3 and SUMO-1 are conjugated by the UBC9 E2 enzyme. Proc Natl Acad Sci USA 1998; 95: 560–4.
110. Pichler A, Gast A, Seeler JS, Dejean A, Melchior F. The nucleoporin RanBP2 has SUMO1 E3 ligase activity. Cell 2002; 108: 109–20.
111. Zhu S, Goeres J, Sixt KM, Bekes M, Zhang XD, Salvesen GS, Matunis MJ. Protection from isopeptidase-mediated deconjugation regulates paralog-selective sumoylation of RanGAP1. Mol Cell 2009; 33: 570–80.
112. Papouli E, Chen S, Davies AA, Huttner D, Krejci L, Sung P, Ulrich HD. Crosstalk between SUMO and ubiquitin on PCNA is mediated by recruitment of the helicase Srs2p. Mol Cell 2005; 19: 123–33.
113. Lin DY, Huang YS, Jeng JC, Kuo HY, Chang CC, Chao TT, Ho CC, Chen YC, Lin TP, Fang HI, Hung CC, Suen CS, Hwang MJ, Chang KS, Maul GG, Shih HM. Role of SUMO-interacting motif in Daxx SUMO modification, subnuclear localization, and repression of sumoylated transcription factors. Mol Cell 2006; 24: 341–54.
114. Zhu J, Zhu S, Guzzo CM, Ellis NA, Sung KS, Choi CY, Matunis MJ. Small ubiquitin-related modifier (SUMO) binding determines substrate recognition and paralog-selective SUMO modification. J Biol Chem 2008; 283: 29405–15.
115. Takahashi H, Hatakeyama S, Saitoh H, Nakayama KI. Noncovalent SUMO-1 binding activity of thymine DNA glycosylase (TDG) is required for its SUMO-1 modification and colocalization with the promyelocytic leukemia protein. J Biol Chem 2005; 280: 5611–21.
116. Ivanov AV, Peng H, Yurchenko V, Yap KL, Negorev DG, Schultz DC, Psulkowski E, Fredericks WJ, White DE, Maul GG, Sadofsky MJ, Zhou MM, Rauscher FJ 3rd. PHD domain-mediated E3 ligase activity directs intramolecular sumoylation of an adjacent bromodomain required for gene silencing. Mol Cell 2007; 28: 823–37.
117. Guervilly J-H, Takedachi A, Naim V, Scaglione S, Chawhan C, Lovera Y, Despras E, Kuraoka I, Kannouche P, Rosselli F, Gaillard PH. The SLX4 complex is a SUMO E3 ligase that impacts on replication stress outcome and genome stability. Mol Cell 2015; 57: 123–37.
118. Tomasi ML, Ramani K, Ryoo M. Ubiquitin-conjugating enzyme 9 phosphorylation as a novel mechanism for potentiation of the inflammatory response. Am J Pathol 2016; 186: 2326–36.
119. Hsieh YL, Kuo HY, Chang CC, Naik MT, Liao PH, Ho CC, Huang TC, Jeng JC, Hsu PH, Tsai MD, Huang TH, Shih HM. Ubc9 acetylation modulates distinct SUMO target modification and hypoxia response. EMBO J 2013; 32: 791–804.
120. Bencsath KP, Podgorski MS, Pagala VR, Slaughter CA, Schulman BA. Identification of a multifunctional binding site on Ubc9p required for Smt3p conjugation. J Biol Chem 2002; 277: 47938–45.
121. Tatham MH, Kim S, Yu B, Jaffray E, Song J, Zheng J, Rodriguez MS, Hay RT, Chen Y. Role of an N-terminal site of Ubc9 in SUMO-1, -2, and -3 binding and conjugation. Biochemistry 2003; 42: 9959–69.
122. Brzovic PS, Lissounov A, Christensen DE, Hoyt DW, Klevit RE. A UbcH5/ubiquitin noncovalent complex is required for processive BRCA1-directed ubiquitination. Mol Cell 2006; 21: 873–80.
123. McKenna S, Moraes T, Pastushok L, Ptak C, Xiao W, Spyracopoulos L, Ellison MJ. An NMR-based model of the ubiquitin-

bound human ubiquitin conjugation complex Mms2.Ubc13. The structural basis for lysine 63 chain catalysis. J Biol Chem 2003; 278: 13151–8.
124. Pichler A, Knipscheer P, Saitoh H, Sixma TK, Melchior F. The RanBP2 SUMO E3 ligase is neither HECT- nor RING-type. Nat Struct Mol Biol 2004; 11: 984–91.
125. Reverter D, Lima CD. Insights into E3 ligase activity revealed by a SUMO-RanGAP1-Ubc9-Nup358 complex. Nature 2005; 435: 687–92.
126. Heaton PR, Deyrieux AF, Bian XL, Wilson VG. HPV E6 proteins target Ubc9, the SUMO conjugating enzyme. Virus Res 2011; 158: 199–208.
127. Ribet D, Hamon M, Gouin E, Nahori MA, Impens F, Neyret-Kahn H, Gevaert K, Vandekerckhove J, Dejean A, Cossart P. Listeria monocytogenes impairs SUMOylation for efficient infection. Nature 2010; 464: 1192–5.
128. Mo YY, Moschos SJ. Targeting Ubc9 for cancer therapy. Expert Opin Ther Targets 2005; 9: 1203–16.
129. Moschos SJ, Smith AP, Mandic M, Athanassiou C, Watson-Hurst K, Jukic DM, Edington HD, Kirkwood JM, Becker D. SAGE and antibody array analysis of melanoma-infiltrated lymph nodes: identification of Ubc9 as an important molecule in advanced-stage melanomas. Oncogene 2007; 26: 4216–25.
130. Wu F, Zhu S, Ding Y, Beck WT, Mo YY. MicroRNA-mediated regulation of Ubc9 expression in cancer cells. Clin Cancer Res 2009; 15: 1550–7.
131. Zhao Z, Tan X, Zhao A, Zhu L, Yin B, Yuan J, Qiang B, Peng X. microRNA-214-mediated UBC9 expression in glioma. BMB Rep 2012; 45: 641–6.
132. Koh EH, Chen Y, Bader DA, Hamilton MP, He B, York B, Kajimura S, McGuire SE, Hartig SM. Mitochondrial Activity in Human White Adipocytes Is Regulated by the Ubiquitin Carrier Protein 9/microRNA-30a Axis. J Biol Chem 2016; 291: 24747–24755(1083-351X (Electronic)).
133. Su YF, Yang T, Huang H, Liu LF, Hwang J. Phosphorylation of Ubc9 by Cdk1 enhances SUMOylation activity. PLoS One 2012; 7: e34250.
134. Novatchkova M, Bachmair A, Eisenhaber B, Eisenhaber F. Proteins with two SUMO-like domains in chromatin-associated complexes: the RENi (Rad60-Esc2-NIP45) family. BMC Bioinformat 2005; 6: 22.
135. Yang K, Moldovan GL, Vinciguerra P, Murai J, Takeda S, D'Andrea AD. Regulation of the Fanconi anemia pathway by a SUMO-like delivery network. Genes Dev 2011; 25: 1847–58.
136. Prudden J, Perry JJ, Arvai AS, Tainer JA, Boddy MN. Molecular mimicry of SUMO promotes DNA repair. Nat Struct Mol Biol 2009; 16: 509–16.
137. Sekiyama N, Arita K, Ikeda Y, Hashiguchi K, Ariyoshi M, Tochio H, Saitoh H, Shirakawa M. Structural basis for regulation of poly-SUMO chain by a SUMO-like domain of Nip45. Proteins 2010; 78: 1491–502.
138. Sollier J, Driscoll RF, Castellucci F, Castellucci FF, Foiani M, Foiani MF, Jackson SP, Jackson SF, Branzei D, Branzei D. The Saccharomyces cerevisiae Esc2 and Smc5-6 proteins promote sister chromatid junction-mediated intra-S repair. Mol Biol Cell 2009; 20: 1671–82(1939-4586 (Electronic)).
139. Hashiguchi K, Ozaki M, Kuraoka I, Saitoh H. Establishment of a human cell line stably overexpressing mouse Nip45 and characterization of Nip45 subcellular localization. Biochem Biophys Res Commun 2013; 430: 72–7.
140. Raffa GD, Wohlschlegel J, Yates JR, 3rd, Boddy MN. SUMO-binding motifs mediate the Rad60-dependent response to replicative stress and self-association. J Biol Chem 2006; 281: 27973–81.
141. Johnson ES, Gupta AA. An E3-like factor that promotes SUMO conjugation to the yeast septins. Cell 2001; 106: 735–44.
142. Kahyo T, Nishida T, Yasuda H. Involvement of PIAS1 in the sumoylation of tumor suppressor p53. Mol Cell 2001; 8: 713–8.
143. Sachdev S, Bruhn L, Sieber H, Pichler A, Melchior F, Grosschedl R. PIASy, a nuclear matrix-associated SUMO E3 ligase, represses LEF1 activity by sequestration into nuclear bodies. Genes Dev 2001; 15: 3088–103.
144. Schmidt D, Muller S. Members of the PIAS family act as SUMO ligases for c-Jun and p53 and repress p53 activity. Proc Natl Acad Sci USA 2002; 99: 2872–7.
145. Kotaja N, Karvonen U, Janne OA, Palvimo JJ. PIAS proteins modulate transcription factors by functioning as SUMO-1 ligases. Mol Cell Biol 2002; 22: 5222–34.
146. Sapetschnig A, Rischitor G, Braun H, Doll A, Schergaut M, Melchior F, Suske G. Transcription factor Sp3 is silenced through SUMO modification by PIAS1. EMBO J 2002; 21: 5206–15.
147. Potts PR, Yu H. Human MMS21/NSE2 is a SUMO ligase required for DNA repair. Mol Cell Biol 2005; 25: 7021–32.
148. Zhao X, Blobel G. A SUMO ligase is part of a nuclear multiprotein complex that affects DNA repair and chromosomal organization. Proc Natl Acad Sci USA 2005; 102: 4777–82.
149. Andrews EA, Palecek J, Sergeant J, Taylor E, Lehmann AR, Watts FZ. Nse2, a component of the Smc5-6 complex, is a SUMO ligase required for the response to DNA damage. Mol Cell Biol 2005; 25: 185–96.
150. Yunus AA, Lima CD. Structure of the Siz/PIAS SUMO E3 ligase Siz1 and determinants required for SUMO modification of PCNA. Mol Cell 2009; 35: 669–82.
151. Gareau JR, Lima CD. The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition. Nat Rev Mol Cell Biol 2010; 11: 861–71.
152. Rytinki MM, Kaikkonen S, Pehkonen P, Jaaskelainen T, Palvimo JJ. PIAS proteins: pleiotropic interactors associated with SUMO. Cell Mol Life Sci 2009; 66: 3029–41.
153. Liu B, Mink S, Wong KA, Stein N, Getman C, Dempsey PW, Wu H, Shuai K. PIAS1 selectively inhibits interferon-inducible genes and is important in innate immunity. Nat Immunol 2004; 5: 891–8.
154. Wong KA, Kim R, Christofk H, Gao J, Lawson G, Wu H. 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 2004; 24: 5577–86.
155. Roth W, Sustmann C, Kieslinger M, Gilmozzi A, Irmer D, Kremmer E, Turck C, Grosschedl R. PIASy-deficient mice display modest defects in IFN and Wnt signaling. J Immunol 2004; 173: 6189–99.
156. Santti H, Mikkonen L, Anand A, Hirvonen-Santti S, Toppari J, Panhuysen M, Vauti F, Perera M, Corte G, Wurst W, Jänne OA, Palvimo JJ. Disruption of the murine PIASx gene results in reduced testis weight. J Mol Endocrinol 2005; 34: 645–54.
157. Tahk S, Liu B, Chernishof V, Wong KA, Wu H, Shuai K. Control of specificity and magnitude of NF-kB and STAT1-mediated gene activation through PIASy and PIAS1 cooperation. Proc Natl Acad Sci USA 2007; 104: 11643–8.
158. Wu CS, Zou L. The SUMO (Small Ubiquitin-like Modifier) Ligase PIAS3 Primes ATR for Checkpoint Activation. J Biol Chem 2016; 291: 279–90.

159. Liu S, Fan Z, Geng Z, Zhang H, Ye Q, Jiao S, Xu X. PIAS3 promotes homology-directed repair and distal non-homologous end joining. Oncol Lett 2013; 6: 1045–8.
160. Galanty Y, Belotserkovskaya R, Coates J, Polo S, Miller KM, Jackson SP. Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. Nature 2009; 462: 935–9.
161. Morris JR, Boutell C, Keppler M, Densham R, Weekes D, Alamshah A, Butler L, Galanty Y, Pangon L, Kiuchi T, Ng T, Solomon E. The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. Nature 2009; 462: 886–90.
162. Zhang C, Yuan X, Yue L, Fu J, Luo L, Yin Z. PIASy interacts with p73alpha and regulates cell cycle in HEK293 cells. Cell Immunol 2010; 263: 235–40.
163. Munarriz E, Barcaroli D, Stephanou A, Townsend PA, Maisse C, Terrinoni A, Neale MH, Martin SJ, Latchman DS, Knight RA, Melino G, De Laurenzi V. PIAS-1 is a checkpoint regulator which affects exit from G1 and G2 by sumoylation of p73. Mol Cell Biol 2004; 24: 10593–610.
164. Lao M, Shi M, Zou Y, Huang M, Ye Y, Qiu Q, Xiao Y, Zeng S, Liang L, Yang X, Xu H. Protein Inhibitor of Activated STAT3 Regulates Migration, Invasion, and Activation of Fibroblast-like Synoviocytes in Rheumatoid Arthritis. J Immunol 2016; 196: 596–606.
165. Castillo-Lluva S, Tatham MH, Jones RC, Jaffray EG, Edmondson RD, Hay RT, Malliri A. SUMOylation of the GTPase Rac1 is required for optimal cell migration. Nat Cell Biol 2010; 12: 1078–85.
166. Leitao BB, Jones MC, Brosens JJ. The SUMO E3-ligase PIAS1 couples reactive oxygen species-dependent JNK activation to oxidative cell death. FASEB J 2011; 25: 3416–25.
167. Ledsaak M, Bengtsen M, Molvaersmyr AK, Fuglerud BM, Matre V, Eskeland R, Gabrielsen OS. PIAS1 binds p300 and behaves as a coactivator or corepressor of the transcription factor c-Myb dependent on SUMO-status. Biochim Biophys Acta 2016; 1859: 705–18.
168. Estruch SB, Graham SA, Deriziotis P, Fisher SE. The language-related transcription factor FOXP2 is post-translationally modified with small ubiquitin-like modifiers. Sci Rep 2016; 6: 20911.
169. Siatecka M, Soni S, Planutis A, Bieker JJ. Transcriptional activity of erythroid Kruppel-like factor (EKLF/KLF1) modulated by PIAS3 (protein inhibitor of activated STAT3). J Biol Chem 2015; 290: 9929–40.
170. Beketaev I, Kim EY, Zhang Y, Yu W, Qian L, Wang J. Potentiation of Tbx5-mediated transactivation by SUMO conjugation and protein inhibitor of activated STAT 1 (PIAS1). Int J Biochem Cell Biol 2014; 50: 82–92.
171. Sharrocks AD. PIAS proteins and transcriptional regulation--more than just SUMO E3 ligases? Genes Dev 2006; 20: 754–8.
172. Palvimo JJ. PIAS proteins as regulators of small ubiquitin-related modifier (SUMO) modifications and transcription. Biochem Soc Trans 2007; 35(Pt 6): 1405–8.
173. Brown JR, Conn KL, Wasson P, Charman M, Tong L, Grant K, McFarlane S, Boutell C. The SUMO Ligase Protein Inhibitor of Activated STAT 1 (PIAS1) is a constituent PML-NB protein that contributes to the intrinsic antiviral immune response to herpes simplex virus 1 (HSV-1). J Virol 2016; 90: 5939–52.
174. Conn KL, Wasson P, McFarlane S, Tong L, Brown JR, Grant KG, Domingues P, Boutell C. Novel role for protein inhibitor of activated STAT 4 (PIAS4) in the restriction of herpes simplex virus 1 by the cellular intrinsic antiviral immune response. J Virol 2016; 90: 4807–26.
175. Lin HY, Tsai CH, Lin C, Yeh WL, Tsai CF, Chang PC, Wu LH, Lu DY. Cobalt Protoporphyrin Upregulates Cyclooxygenase-2 Expression Through a Heme Oxygenase-Independent Mechanism. Mol Neurobiol 2016; 53: 4497–508.
176. Trinath J, Holla S, Mahadik K, Prakhar P, Singh V, Balaji KN. The WNT signaling pathway contributes to dectin-1-dependent inhibition of Toll-like receptor-induced inflammatory signature. Mol Cell Biol 2014; 34: 4301–14.
177. Liu B, Yang Y, Chernishof V, Loo RR, Jang H, Tahk S, Yang R, Mink S, Shultz D, Bellone CJ, Loo JA, Shuai K. Proinflammatory stimuli induce IKKalpha-mediated phosphorylation of PIAS1 to restrict inflammation and immunity. Cell 2007; 129: 903–14.
178. Liu Y, Zhang YD, Guo L, Huang HY, Zhu H, Huang JX, Liu Y, Zhou SR, Dang YJ, Li X, Tang QQ. Protein inhibitor of activated STAT 1 (PIAS1) is identified as the SUMO E3 ligase of CCAAT/enhancer-binding protein beta (C/EBPbeta) during adipogenesis. Mol Cell Biol 2013; 33: 4606–17.
179. Tatham MH, Kim S, Jaffray E, Song J, Chen Y, Hay RT. Unique binding interactions among Ubc9, SUMO and RanBP2 reveal a mechanism for SUMO paralog selection. Nat Struct Mol Biol 2005; 12: 67–74.
180. Wu J, Matunis MJ, Kraemer D, Blobel G, Coutavas E. Nup358, a cytoplasmically exposed nucleoporin with peptide repeats, Ran-GTP binding sites, zinc fingers, a cyclophilin A homologous domain, and a leucine-rich region. J Biol Chem 1995; 270: 14209–13.
181. Yokoyama N, Hayashi N, Seki T, Pante N, Ohba T, Nishii K, Kuma K, Hayashida T, Miyata T, Aebi U. A giant nucleopore protein that binds Ran/TC4. Nature 1995; 376: 184–8.
182. Chen JW, Barker AR, Wakefield JG. The ran pathway in drosophila melanogaster mitosis. Front Cell Dev Biol 2015; 3: 74.
183. Salina D, Enarson P, Rattner JB, Burke B. Nup358 integrates nuclear envelope breakdown with kinetochore assembly. J Cell Biol 2003; 162: 991–1001.
184. Joseph J, Liu ST, Jablonski SA, Yen TJ, Dasso M. The RanGAP1-RanBP2 complex is essential for microtubule-kinetochore interactions in vivo. Curr Biol 2004; 14: 611–7.
185. Swaminathan S, Kiendl F, Korner R, Lupetti R, Hengst L, Melchior F. RanGAP1*SUMO1 is phosphorylated at the onset of mitosis and remains associated with RanBP2 upon NPC disassembly. J Cell Biol 2004; 164: 965–71.
186. Aslanukov A, Bhowmick R, Guruju M, Oswald J, Raz D, Bush RA, Sieving PA, Lu X, Bock CB, Ferreira PA. RanBP2 modulates Cox11 and hexokinase I activities and haploinsufficiency of RanBP2 causes deficits in glucose metabolism. PLoS Genet 2006; 2: e177.
187. Dawlaty MM, Malureanu L, Jeganathan KB, Kao E, Sustmann C, Tahk S, Shuai K, Grosschedl R, van Deursen JM. Resolution of sister centromeres requires RanBP2-mediated SUMOylation of topoisomerase IIa. Cell 2008; 133: 103–15.
188. Clarke DJ, Johnson RT, Downes CS. Topoisomerase II inhibition prevents anaphase chromatid segregation in mammalian cells independently of the generation of DNA strand breaks. J Cell Sci 1993; 105 (Pt 2): 563–9.
189. Abascal F, Tress ML, Valencia A. Alternative splicing and co-option of transposable elements: the case of TMPO/LAP2alpha and ZNF451 in mammals. Bioinformatics 2015; 31: 2257–61.
190. Karvonen U, Jaaskelainen T, Rytinki M, Kaikkonen S, Palvimo JJ. ZNF451 is a novel PML body- and SUMO-associated transcriptional coregulator. J Mol Biol 2008; 382: 585–600.

191. Feng Y, Wu H, Xu Y, Zhang Z, Liu T, Lin X, Feng XH. Zinc finger protein 451 is a novel Smad corepressor in transforming growth factor-b signaling. J Biol Chem 2014; 289: 2072–83.
192. Koidl S, Eisenhardt N, Fatouros C, Droescher M, Chaugule VK, Pichler A. The SUMO2/3 specific E3 ligase ZNF451-1 regulates PML stability. Int J Biochem Cell Biol 2016; 79: 478–87.
193. Gareau JR, Reverter D, Lima CD. Determinants of small ubiquitin-like modifier 1 (SUMO1) protein specificity, E3 ligase, and SUMO-RanGAP1 binding activities of nucleoporin RanBP2. J Biol Chem 2012; 287: 4740–51.
194. Werner A, Flotho A, Melchior F. The RanBP2/RanGAP1*SUMO1/Ubc9 complex is a multisubunit SUMO E3 ligase. Mol Cell 2012; 46: 287–98.
195. Eisenhardt N, Chaugule VK, Pichler A. A Fluorescent In Vitro Assay to Investigate Paralog-Specific SUMO Conjugation. Methods Mol Biol 2016; 1475: 67–78.
196. Fujiwara K, Hasegawa K, Oka M, Yoneda Y, Yoshikawa K. Terminal differentiation of cortical neurons rapidly remodels RanGAP-mediated nuclear transport system. Genes Cells 2016; 21: 1176–94.
197. Psakhye I, Jentsch S. Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. Cell 2012; 151: 807–20.
198. Maison C, Bailly D, Quivy JP, Almouzni G. The methyltransferase Suv39h1 links the SUMO pathway to HP1a marking at pericentric heterochromatin. Nat Commun 2016; 7: 12224.
199. Reindle A, Belichenko I, Bylebyl GR, Chen XL, Gandhi N, Johnson ES. Multiple domains in Siz SUMO ligases contribute to substrate selectivity. J Cell Sci 2006; 119(Pt 22): 4749–57.
200. Pichler A. Analysis of sumoylation. Methods Mol Biol 2008; 446: 131–8.
201. Ritterhoff T, Das H, Hofhaus G, Schroder RR, Flotho A, Melchior F. The RanBP2/RanGAP1*SUMO1/Ubc9 SUMO E3 ligase is a disassembly machine for Crm1-dependent nuclear export complexes. Nat Commun 2016; 7: 11482.
202. Swatek KN, Komander D. Ubiquitin modifications. Cell Res 2016; 26: 399–422.
203. Poulsen SL, Hansen RK, Wagner SA, van Cuijk L, van Belle GJ, Streicher W, Wikström M, Choudhary C, Houtsmuller AB, Marteijn JA, Bekker-Jensen S, Mailand N. RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response. J Cell Biol 2013; 201: 797–807.
204. Ouyang J, Garner E, Hallet A, Nguyen HD, Rickman KA, Gill G, Smogorzewska A, Zou L. Noncovalent interactions with SUMO and ubiquitin orchestrate distinct functions of the SLX4 complex in genome maintenance. Mol Cell 2015; 57: 108–22.
205. Cheng CH, Lo YH, Liang SS, Ti SC, Lin FM, Yeh CH, Huang HY, Wang TF. SUMO modifications control assembly of synaptonemal complex and polycomplex in meiosis of Saccharomyces cerevisiae. Genes Dev 2006; 20: 2067–81.
206. Srikumar T, Lewicki MC, Costanzo M, Tkach JM, van Bakel H, Tsui K, Johnson ES, Brown GW, Andrews BJ, Boone C, Giaever G, Nislow C, Raught B. Global analysis of SUMO chain function reveals multiple roles in chromatin regulation. J Cell Biol 2013; 201: 145–63.
207. Plechanovova A, Jaffray EG, Tatham MH, Naismith JH, Hay RT. Structure of a RING E3 ligase and ubiquitin-loaded E2 primed for catalysis. Nature 2012; 489: 115–20.
208. Pruneda JN, Littlefield PJ, Soss SE, Nordquist KA, Chazin WJ, Brzovic PS, Klevit RE. Structure of an E3:E2~Ub complex reveals an allosteric mechanism shared among RING/U-box ligases. Mol Cell 2012; 47: 933–42.
209. Namanja AT, Li YJ, Su Y, Wong S, Lu J, Colson LT, Wu C, Li SS, Chen Y. Insights into high affinity small ubiquitin-like modifier (SUMO) recognition by SUMO-interacting motifs (SIMs) revealed by a combination of NMR and peptide array analysis. J Biol Chem 2012; 287: 3231–40.