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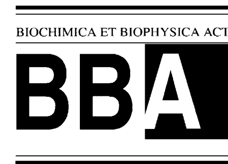

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Review

SUMO protein modification

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Abstract

SUMO (*s*mall *u*biquitin-related *mo*difier) family proteins are not only structurally but also mechanistically related to ubiquitin in that they are posttranslationally attached to other proteins. As ubiquitin, SUMO is covalently linked to its substrates via amide (isopeptide) bonds formed between its C-terminal glycine residue and the ε-amino group of internal lysine residues. The enzymes involved in the reversible conjugation of SUMO are similar to those mediating the ubiquitin conjugation. Since its discovery in 1996, SUMO has received a high degree of attention because of its intriguing and essential functions, and because its substrates include a variety of biomedically important proteins such as tumor suppressor p53, c-jun, PML and huntingtin. SUMO modification appears to play important roles in diverse processes such as chromosome segregation and cell division, DNA replication and repair, nuclear protein import, protein targeting to and formation of certain subnuclear structures, and the regulation of a variety of processes including the inflammatory response in mammals and the regulation of flowering time in plants.
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Keywords: SUMO; Protein; Modification

1. Introduction

Posttranslational protein modifications are versatile devices that cells use to control the function of proteins by regulating their activity, subcellular localization, stability, as well as their interaction with other proteins. The reversibility of protein modifications enables the participation of proteins regulated by them in multiple rounds of functional circuits. Protein modifications are also important to rapidly regulate and orchestrate protein functions in response to changes in a cell's state or its environment, without altering their synthesis or turnover rates. Ubiquitin-related protein modifiers, collectively termed Ubls, are posttranslationally attached to substrate proteins by enzymatic reactions that are similar to ubiquitin conjugation [1,2]. While some Ubls such as Rub1/NEDD8 display a high degree of sequence similarity to ubiquitin, others such as SUMO do not. Due to its involvement in a variety of important processes of eukaryotic cell biology, SUMO is maybe the most intriguing Ubl. Despite the similarities in their structure and the enzymatic reactions underlying their conjugation, SUMO and ubiquitin have distinct nonoverlapping functions. There are, however, examples of substrates such as IκBα and PCNA that can be alternatively conjugated to either modifier.

A number of excellent reviews with more detailed discussions of earlier studies and closer looks at various functional aspects of SUMO research, as well as at the evolution of Ubl families, are recommended for further readings [3–13]. This review attempts to summarize the current status of a rapidly increasing knowledge of the mechanisms and functions of SUMO systems in various eukaryotic model organisms with an emphasis on the enzymes mediating the SUMO cycle, and on the most recent discoveries on SUMO targets.

2. Discovery of SUMO protein modification

The ubiquitin-related protein SUMO-1 was discovered in studies on nuclear import in mammalian cells as a covalent modification of RanGAP1. This discovery may have been facilitated by its unique property of being nearly quantitatively and constitutively modified with SUMO. This

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modification targets the otherwise cytosolic RanGAP1 to the nuclear pore complex (NPC) where it participates in nuclear import by activating the GTPase activity of the cytosol/nucleus shuttling factor Ran [14–19]. Sumoylation of RanGAP1 leads to its interaction with the Ran binding protein RanBP2 at the cytoplasmic filaments of the NPC. As discussed in a later section, RanBP2 itself is modified by sumoylation and, moreover, has recently been shown to act as a SUMO ligase [19,20]. SUMO was independently identified in a variety of studies explaining why in the literature it also appears as 'GMP', 'PIC1', 'sentrin', 'SMT3', or 'UBL1' [14,21–24].

3. SUMO isoforms and structure

SUMO is encoded by single genes in yeast species and invertebrates. Whereas the SUMO-encoding *SMT3* gene is essential in *Saccharomyces cerevisiae* [25], its counterpart *pmt3* in *Schizosaccharomyces pombe* is not [26]. *Pmt3*$^{-}$ mutants, however, grow poorly and display various phenotypes that have also been associated with conditional mutations in the SUMO system of *S. cerevisiae*.

Four different SUMO isoforms termed SUMO-1, SUMO-2, SUMO-3, and SUMO-4 have been detected in mammals. SUMO-2 and SUMO-3 are very similar in sequence and therefore sometimes termed SUMO-2/3 in one breath. The divergence of the functions of these isoforms is just beginning to emerge. SUMO-1, which displays a slightly higher degree of similarity to yeast SUMO/Smt3 (~47% identical residues), seems to be the most prominently conjugated isoform under normal conditions. SUMO-2/3 (~45% of the residues identical to those of Smt3) appears to be preferentially conjugated to proteins under stress conditions such as increased temperature [27]. There are, however, examples of substrates such as topoisomerase II and CAAT/enhancer-binding protein-beta (C/EBPβ) that are specifically modified by SUMO-2/3 under normal physiological conditions [28,29]. The very recently identified fourth isoform, SUMO-4, is encoded by a sequence that lies within an intron of the human TAB2 gene [30]. The expression of this gene is strongest in kidney cells. While SUMO-2, SUMO-3, and SUMO-4 share a SUMO attachment consensus site (see below), such a site is absent from SUMO-1. Consistent with this observation, in contrast to SUMO-1, SUMO-2/3 as well as SUMO-4 have been shown to form SUMO chains in vitro and in vivo [30,31]. Multiple SUMO isoforms, eight to be exact, are encoded by the genome of the model plant *Arabidopsis thaliania*. Similar to mammals, the conjugation of certain isoforms (SUMO1 and SUMO2) is induced when *Arabidopsis* is subjected to heat stress [32].

The structure of human SUMO-1 has been determined by NMR and compared to the crystal structure of ubiquitin [33]. More recently, the structure of budding yeast SUMO (Smt3) has been determined after co-crystalization with

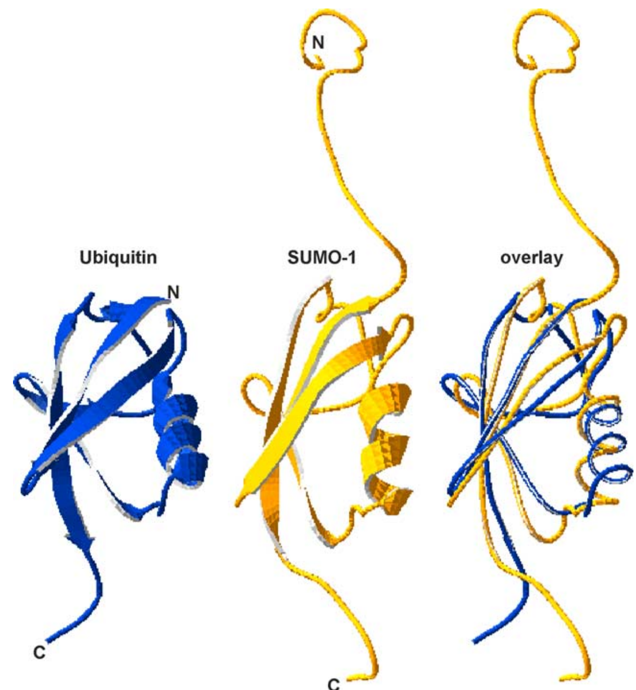

Fig. 1. Structure comparison of ubiquitin and human SUMO-1. Both proteins share a characteristic tightly packed ββαββαβ fold, and a C-terminal di-glycine motif. SUMO is distinguished by a long and flexible N-terminal extension. The structure of ubiquitin was determined by X-ray crystallography [185]; the structure of SUMO in solution by NMR [33].

Ulp1 [34], and in solution with and without Ubc9 [35]. Although the sequence identity between SUMO and ubiquitin is relatively low (~18% identity) the overall three-dimensional structures are very similar (Fig. 1). The surface charge distributions of the two proteins, however, are quite different, indicating that they interact specifically with distinct enzymes and substrates (see below). Another prominent feature of SUMO-1 is a protruding long and flexible N-terminal domain, which is absent in ubiquitin. In yeast, a lysine residue within this N-terminal domain has been implicated in the formation of poly-SUMO chains [36,37]. Surprisingly, however, the entire extension including this lysine can be deleted without severe consequences for the yeast indicating that, in contrast to ubiquitin, chain formation is not important for SUMO function in *S. cerevisiae* [36]. A feature that is shared between the mature forms of SUMO and ubiquitin, and also some other Ubls, is a di-glycine motif at the C-terminus. This motif was shown to be critical for SUMO conjugation in *S. cerevisiae* [25].

4. Substrate selection and SUMO conjugation

The analysis of an increasing number of SUMO targets has confirmed that the majority of SUMO accepting lysine residues (K) lie within the consensus sequence ΨKXE. Ψ is an aliphatic residue, preferably L, I or V [13]. It was

moreover shown that this sequence constitutes a transferable motif sufficient to transform test proteins into suitable substrates for sumoylation in vitro [38]. In the same study it was shown, however, that the same test proteins required a nuclear targeting sequence to be sumoylated in vivo. In another study it was shown that the sumoylation consensus sequence mediates direct interaction with SUMO-conjugating enzyme Ubc9 [39]. It is assumed that this interaction is sufficient to target some substrates such as RanGAP, whereas SUMO ligases are required in addition for modification of others (see below). Within the latter group of substrates are probably those that are sumoylated at nonconsensus sites.

5. Enzymes mediating the SUMO cycle

Sumoylation appears to be a highly selective process both with respect to the choice of substrates as well as to the timing of their modification. How substrate specificity and the timing of their modification are achieved is beginning to emerge. The enzymes required for reversible SUMO conjugation (sumoylation) were first characterized in the yeast *S. cerevisiae* (Fig. 1). Some of these enzymes such as the SUMO-activating enzyme (E1) and SUMO-conjugating enzyme (E2) have sequences with similarities to their counterparts in the ubiquitin system, and are conserved from yeast to humans. Despite the similarity to enzymes of the ubiquitin system or to those involved in conjugation of other Ubls such as Rub1/NEDD8, the enzymes mediating the SUMO cycle appear to be specific for this modifier. The genes for several enzymes of the SUMO modification system, such as E1, E2, and Ulp1, as well as the gene encoding SUMO itself are essential for viability in bakers' yeast. Results obtained for the E1 and E2 enzymes established that SUMO modification is an essential process in higher eukaryotes as well. Genetic analyses in *S. cerevisiae* have revealed that a balance between the SUMO conjugating and deconjugating activities is critical for a variety of processes. These findings were consistent with the general observation that in cell populations and probably within a single cell only a small fraction of a given substrate is to be detected in its sumoylated form at a given time. In other words, the sumoylation/desumoylation cycle is highly dynamic, and is for many substrates synchronized with the cell cycle. How this synchronicity is achieved is largely unknown. The phenotypes of *S. cerevisiae* mutants with conditional defects in genes of the SUMO cycle have uncovered the essential role of SUMO modification in the cell division cycle. The relevant SUMO targets underlying this function, however, are still largely unknown as well. Several enzymes of the SUMO system including E1, E2, some E3s and deconjugating enzymes (Ulp2), as well as most SUMO conjugates are found enriched within the cell nucleus, whereas the deconjugating enzymes Ulp1 in *S. cerevisiae* and SENP2 in mammals as well as the SUMO ligase RanBP2 are associated with the nuclear pore [40–42]. The latter distribution is consistent with functions of sumoylation in cytosol/nucleus transit.

6. SUMO-activating enzyme (E1)

Like ubiquitin, the C-terminus of mature SUMO generated by the activity of processing protease (discussed in a later section) needs to be activated for posttranslational conjugation, i.e., for isopeptide bond formation with substrate lysine residues. SUMO-activating enzyme (E1) is a heterodimer of Aos1 (SAE1, Sua1) and Uba2 (SAE2), proteins with sequence similarities to the N- and C-terminal parts, respectively, of ubiquitin-activating enzymes [25,43–46]. ATP-dependent activation occurs via a noncovalently bound SUMO adenylate intermediate followed by the formation of a thioester between SUMO and an active site cysteine in Uba2 [13] (Fig. 2). In *S. cerevisiae*, both

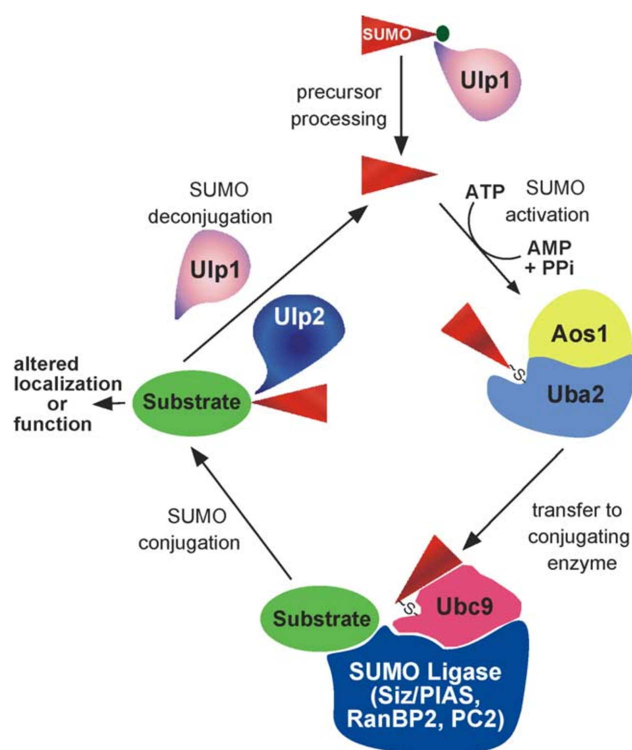

Fig. 2. SUMO conjugation/deconjugation cycle. SUMO/Smt3 is synthesized as a precursor with a short C-terminal extension, which in *S. cerevisiae* is cleaved off by the processing protease Ulp1. For conjugation, the C-terminus of mature SUMO needs first to be activated by the SUMO-activating enzyme (E1), a heterodimeric protein composed of Uba2 and Aos1. Activation is ATP-dependent, proceeds via a SUMO-adenylate intermediate, and results in SUMO being linked by a thioester to a cysteine residue in Uba2. Activated SUMO is then transferred, in a transesterification reaction, to a cysteine residue of Ubc9, a conjugating enzyme (E2) specific for SUMO. In conjunction with the substrate recognizing SUMO ligases (E3), Ubc9 conjugates SUMO to a variety of substrate proteins. Sumoylated substrates are deconjugated by two isopeptidase activities residing in the Ulp1 and Ulp2 proteases.

subunits of E1 are essential for viability consistent with an essential function of SUMO modification [25,43]. In *S. pombe*, in which SUMO conjugation is not essential for viability, however, residual cellular SUMO conjugates were to be detected in mutants lacking the Aos1 orthologue Rad31. These data suggested that Fub1, the orthologue of Uba2, may have a low activity in the absence of its partner subunit [47]. An alternative explanation, which is consistent with the lack of in vitro activity of the *S. cerevisiae* Uba2 in the absence of Aos1 [25], would be that Aos1 paralogues involved in activating other Ubls such as Rub1 inefficiently substitute for Rad31. Interestingly, levels of human Aos1 were found to be regulated during the cell cycle reaching a peak in S phase, whereas Uba2 levels remained unchanged [46]. These data suggested that regulation of SUMO-activating enzyme might be mediated via Aos1. This assumption is consistent with the presence of two genes, SAE1a and SAE1b, coding for Aos1-type subunits with ~81% sequence identity to each other in the *Arabidopsis* genome, with only one gene (SAE2) present coding for Uba2 [32]. The heterodimeric design of activating enzymes for several UBLs appears to be evolutionary linked to a homodimeric bacterial enzyme involved in molybdenum cofactor biosynthesis. The homodimeric MoeB protein activates the C terminus of MoaD for its transfer to a sulfur atom [48]. MoaD is structurally related to UBLs also bearing a C-terminal di-glycine motif. MoeB shares sequence elements with Aos1 as well as with Uba2 and its crystal structure displays similarities with the NEDD8/Rub1 activating enzyme [48,49]. SUMO-activating enzyme is found predominantly in the cell nuclei of species ranging from yeast to mammals [43,46,50]. The *Drosophila* Uba2 is expressed at all times of the life cycle but is most abundant during embryogenesis, suggesting a requirement for higher sumoylation rates in proliferating cells [50].

7. SUMO-conjugating enzyme (E2)

In a transesterification reaction, activated SUMO is transferred from the Uba2 subunit of SUMO-activating enzyme to a single SUMO-conjugating enzyme (E2) known as Ubc9 (Fig. 2). As a result, a SUMO-Ubc9 thioester intermediate is formed [51–53]. In most species studied, the genes encoding Ubc9 are essential, the exception again being *S. pombe*, in which SUMO conjugation is not required for viability [13,54–56]. As the SUMO-activating enzyme, Ubc9 is a predominantly nuclear protein [54]. Studies on Ubc9 in mammals have shown that at least a fraction of it is associated with cytoplasmic and nucleoplasmic filaments of the NPC [42].

Ubc9 proteins are well conserved with ~56% identity between the mammalian and *S. cerevisiae* orthologues. The mammalian version (mouse and human Ubc9 proteins are identical), moreover, has been shown to be capable of substituting for the essential functions of its counterpart in *S. cerevisiae* [57]. Crystal structure analysis of murine/human Ubc9 established that it has an overall fold that is quite similar to the core domain of ubiquitin-conjugating enzymes [58,59]. The structure of Ubc9, however, exhibits various differences to its homologues mediating ubiquitylation in surface charge distribution and the presence of small insertions, which appear to be critical for interactions with SUMO, the SUMO-specific E1, and with its substrates [60–62]. Mapping of binding sites of the mammalian SUMO paralogues SUMO-1 and SUMO-2/3 revealed that Ubc9 does not discriminate between them [63]. Sites for noncovalent binding of SUMO in the N-terminus of Ubc9, interestingly, appear to overlap with those for E1 [37]. Increasing concentrations of SUMO were shown to result in a displacement of E1 from E1–Ubc9 complexes in vitro with the concomitant formation of a noncovalent Ubc9–SUMO complex [37]. The physiological relevance of this noncovalent binding site for SUMO on Ubc9 is unclear. It may increase the affinity of a SUMO thioester-bound Ubc9 to the distal end of a growing poly-SUMO chain [37]. As mentioned above, Ubc9 can bind at least a subset of its substrate via their sumoylation consensus sequences (ΨKXE) [39]. Determination of the crystal structure of human Ubc9 in a complex with a C-terminal fragment of mouse RanGAP revealed that the Lys residue within this motif comes to lie in a shallow, mainly hydrophobic groove of Ubc9. Within this groove, Asp127 of Ubc9 appears to engage in hydrogen bonding with the Lys residue, which may assist in catalysis. A number of van der Waals interactions between residues in Ubc9 and the hydrophobic (Ψ) residue as well as the acidic (E) residue in the consensus motif stabilize the interaction. As residues immediately preceding or following the consensus motif are not in direct contact with Ubc9, this four-amino-acid sequence appears to be sufficient for binding in this groove [64]. Beyond these interactions, RanGap appears to bear additional Ubc9 binding sites, which may explain why RanGap can by sumoylated in the absence of SUMO ligases [64]. It is worth noting in this context that RanGAP is an unusual SUMO target in that it appears to be by and large constitutively and quantitatively modified. The additional Ubc9 binding sites in RanGAP may serve as a "built in" E3 like property enabling modification with high efficiency [65]. Another role of Ubc9 has been reported that apparently does not require its enzymatic function as an E2. Ubc9 mediates nuclear targeting of homeobox protein Vsx-1 via binding to its nuclear localization signal [66].

8. SUMO ligases (E3)

While E1 and E2 were shown to be sufficient for sumoylation of various substrates in vitro, recent studies

have demonstrated that in vivo at least the majority of SUMO targeting reactions require E3 activity. Three types of SUMO ligases have been described PIAS, RanBP2 and PC2, all of which interact with Ubc9 and enhance sumoylation both in vivo and in vitro.

8.1. Siz/PIAS

The prototypes of the PIAS class of SUMO ligases were discovered in yeast. The related proteins Siz1/Ull1 and Siz2/Nfi1 were shown to mediate the majority of sumoylation in *S. cerevisiae* [67,68]. Surprisingly, however, *siz1 siz2* double null mutants are viable, albeit with a reduced ability to divide properly in particular at lower temperatures [67]. It can be concluded that either Ubc9 is sufficient to execute the essential function of SUMO conjugation in the absence of these enzymes, or additional unrecognized SUMO ligases are present in *S. cerevisiae*. Siz1 was shown to mediate sumoylation of yeast septins in vivo and in vitro, and of PCNA in vivo [67,69,70]. Overexpression of Siz2 was reported to enhance sumoylation of Pds5 [71]. In all of these cases, modification appears to be cell cycle-regulated. Septins on the mother side of the bud neck become sumoylated when cells enter mitosis. Siz1 is localized to the mother side of the bud neck as well during this period, which appears to be regulated by phosphorylation [67]. PCNA is modified during S phase; how this is regulated is unclear [70]. Pds5 sumoylation is highest during mitosis. Whether regulation of the sumoylation reaction is involved in the control of this timing is not known [71].

Johnson and Gupta [67] noted that Siz1 and Siz2 share an area of sequence homology with proteins of the conserved PIAS (*p*rotein *i*nhibitor of *a*ctivated *S*TAT) family. The region of homology includes a segment that resembles the RING domains of ubiquitin ligases and was therefore dubbed SP-RING (Siz/PIAS RING) [67,72,73]. This domain binds to Ubc9 and is required for E3 activity [68,74]. The identification and characterization of SP-RING-containing proteins in yeast spurred a number of studies that characterized PIAS proteins as SUMO ligases.

Mammalian cells contain at least four PIAS proteins, PIAS1, 3, x and y, which are negative regulators of the cytokine-mediated JAK-STAT signaling pathway [75] (reviewed in Ref. [76]). PIASx comes in two isoforms apparently resulting from alternative splicing, PIASxα/ARIP3 (*a*ndrogen *r*eceptor *b*inding *p*rotein) and PIASxβ/Miz1 (*M*sx2-*i*nteracting *z*inc finger) [75,77,78]. PIAS1 and PIAS3 bind to STAT1 and STAT3 (*s*ignal *t*ransducers and *a*ctivators of *t*ranscription), respectively, and inhibit their binding to DNA [75,79]. In contrast, PIASx and PIASy, which inhibit STAT1, 3 and 4, do not appear to inhibit the binding of their target STATs to DNA [78]. PIASxα and PIAS3 have been shown to enhance sumoylation of STAT1 on a single residue (Lys703) in vivo and in vitro; PIAS1 did so to a lesser extent [80,81]. Expression of a STAT1 K703R mutant resulted in an increased response of cells to interferon-γ, indicating that SUMO modification of this site serves to down-regulate this response [81]. PIASy was shown to negatively regulate transcription factors LEF1 and SMAD3 by sumoylation [82,83]. PIAS1 and PIASxα were shown to promote sumoylation of SMAD4. Apart of these examples, a growing group of transcription factors (see Table 1), the activities of which were shown to be modulated by PIAS-mediated sumoylation includes c-jun, p53, mdm2, Sp3, SATB2, C/EBPβ, Tcf-4 and androgen receptor [84–90]. A recent potential addition to this family of ligases is hZIMP10, a PIAS-like protein that may act as a SUMO ligase for androgen receptor [91].

PIAS proteins, in addition, appear to influence the activity of transcription factors by mechanisms independent of their activity as SUMO ligases either by inhibiting their binding to DNA, by acting as co-repressors or co-activators, by recruiting histone deacetylase (HDAC), or by sequestering them to nuclear bodies [82,92] (for more detailed reviews, see Refs. [8,10,11,93,94]). The latter function is consistent with the observation that PIAS proteins display divergent distribution in nuclear speckles [74]. PIAS proteins are themselves subject to sumoylation. They also bind to and thereby tether other sumoylated proteins [74].

In summary, PIAS proteins constitute a family of proteins with complex functions that are mediated either by virtue of their SUMO ligase activity or by interaction with unmodified or SUMO-modified proteins, most of which are transcription factors.

8.2. RanBP2

A second type of SUMO E3 is the nucleoporin RanBP2/Nup358 [20]. It does not display an obvious sequence relation to PIAS-type SUMO ligases or ubiquitin ligases. RanBP2 (*Ran b*inding *p*rotein 2) is located at the cytoplasmic filaments of the NPC, where it interacts with sumoylated RanGAP and the GTPase Ran. RanBP2 was itself one of the first SUMO targets to be identified [19]. Using a YFP-SUMO construct, Pichler et al. [20] detected a high sumoylation activity linked to the nuclear envelope. Further characterization revealed that this SUMO E3 activity resided in a ~30-kDa domain of RanBP2 which is characterized by the presence of two internal repeats of ~50 residues. This domain in the presence of E1, E2, SUMO and ATP was sufficient to mediate in vitro sumoylation of Sp100 and HDAC4 [20,95]. Based on these findings, a model was proposed, in which RanBP2 constitutes an NPC-associated SUMO ligase that couples sumoylation with nuclear import [20,96].

Recent studies indicate that the complex formed between RanBP2 and sumoylated RanGAP remains intact when the nuclear envelope breaks down during mitosis. A role of RanBP2 and this complex in the maturation of kinetochores and their attachment to microtubules in this phase of the cell

Table 1
SUMO targets

SUMO target	Effect of SUMO modification	Reference
In yeast		
Septins	unclear, potentially involved in reorganization of septin ring structure	[125,186]
Top1	induced by Top1 inhibitor camptothecin,	[187]
Top2	regulates function of Top2 that controls centromeric cohesion	[168]
PCNA	unclear, possible role in DNA replication	[70]
Pds5	promotes dissolution of chromatid cohesion	[71]
In Drosophila		
Tramtrack 69	unclear	[188]
Neuronal CaMKII	unclear	[189]
In mammals		
RanGAP	Targeting to NPC, interaction with RanBP2	[14,15]
IκBα	prevents ubiquitylation	[163]
NEMO/IKKγ	mediates genotoxic stress-induced nuclear localization	[133]
Axin	probably required for the activation of JNK	[190]
PML	localization to and formation of nuclear PML bodies/ND10	[138]
SP100, Daxx	unclear, not essential for localization to ND10	[138,150]
Topors	unknown, not required for localization to ND10	[155]
LEF1	not essential for targeting to nuclear bodies	[82]
TEL/ETV6	localization to intranuclear 'TEL bodies'	[158]
SMAD4	targeting to nuclear speckles, regulation of stability	[87,191,192]
HIPK2	targeted to nuclear speckles	[193]
Thymine-DNA glycosylase	induces dissociation from abasic sites	[174]
SP3	inhibition, targeting to nuclear envelope and nuclear dots	[86,153]
TOPI, TOPII	inducible by inhibitors	[187,194,195]
RAD51	may regulate role in recombination	[47,57,196,197]
DNA methyltrans-ferase Dnmt3b	unclear	[198]
Histone H4	silencing by recruitment of histone deacetylase and heterochromatin protein 1	[161]
HDAC1, HDAC4	Enhances HDAC1-mediated repression of transcription	[95,162]

Table 1 (*continued*)

SUMO target	Effect of SUMO modification	Reference
Elk-1	recruits HDAC for transcriptional repression of Elk-1 target genes	[160]
p300	inhibits transcription activation potential by mediating binding to HDAC6	[199]
p53	enhances transcriptional activation	[175,176]
p73	may affect subcellular localization	[200]
c-Myb	increases stability and negatively regulates transactivation function	[201]
c-jun	negative regulation of transcription activation potential	[84]
Progesterone receptor (PR)	SUMO-1 overexpression enhances PR-mediated transcription.	[202]
Androgen receptor (AR	inhibition of transactivation potential	[90,203]
GRIP1	inhibits interaction and colocalization with androgen receptor	[204]
Glucocorticoid receptor	regulates 'synergy control' of transcriptional activation potential and turnover	[205]
GATA-2	suppresses GATA-2-dependent activation of endothelial cell-specific genes	[206]
AP-2	suppresses transcription activation potential	[207]
SCREP	suppresses transactivation capacity	[208]
IRF-1	suppresses transcription activation potential	[209]
Serum response factor	suppresses transcription activation potential	[210]
SATB2	enhances transcription activation and localization to matrix attachment regions	[211]
STAT1	suppresses IFNγ-induced transactivation potential	[81]
C/EBPβ	enhances ability to repress transcription of certain genes	[28]
Nurr1	enhances transcription activation potential	[212]
Tcf-4	enhances β catenin-dependent transcriptional activation by Tcf-4	[89]
PLZF	enhances DNA binding activity and capacity to repress transcription	[213]
HSF1	regulates activity, stressed-induced and phosphorylation-dependent	[214–216]

Table 1 (*continued*)

SUMO target	Effect of SUMO modification	Reference
HSF2	regulates DNA binding activity	[156]
CREB	hypoxia-induced, may stabilize CREB and promote nuclear localization	[132]
Pdx1	nuclear localization and insulin gene activation	[217]
CtBP	nuclear localization enabling transcriptional repression	[105]
C/EBPs	suppresses inhibitory effect of regulatory domain in C/EBPε, blocks transcriptional synergy control element of C/EBPα	[88,218]
ARNT	affects interaction with other transcription factors	[219]
Focal adhesion kinase	enhances autophosphorylation activity	[220]
GLUT1, GLUT4	regulation of abundance of these transporters	[221]
Adenoviral E1B	cytosol to nucleus relocalization, required for oncogenic transformation	[131]
Bovine papillomavirus E1	intranuclear distribution, viral replication	[222]
Cytomegalovirus IE2-82	enhances transactivation capacity	[223]

cycle has been demonstrated recently [97,98]. The RanBP2/RanGap-SUMO complex is relocalized to kinetochores during mitosis in a microtubule- and SUMO-dependent manner [98,99]. Cell cycle-dependent phosphorylation may be involved in regulating this processes [100].

8.3. PC2

The polycomb group (PcG) protein PC2 was recently reported to be a third type of SUMO ligase, which appears to be structurally unrelated to Siz/PIAS, or RanBP2 and to ubiquitin ligases [101]. PcG proteins form large multimeric complexes, which are microscopically detectable as discrete foci, so-called PcG bodies, within cell nuclei. PcG proteins comprise a family of functionally related proteins, which were originally identified in *Drosophila* as negative regulators of homeotic gene expression (for review see Ref. [102]). PcG proteins mediate transcriptional silencing of these genes by modifying histones [103,104] The human PcG protein PC2 recruits the transcriptional co-repressor CtBP as well as Ubc9 to PcG bodies, and stimulates CtBP sumoylation in vivo and in vitro. CtBP was also found to be modified by PIAS1 and PIASxβ in vitro [105]. Similar to PIAS and RanBP2, also PC2 itself is modified by sumoylation [101]. Recent studies in *C. elegans* provided genetic evidence for a role of sumoylation in the regulation of homeotic Hox genes via the PcG protein SOP-2 [106].

9. SUMO deconjugating and precursor processing enzymes

SUMO cleaving enzymes play important roles at two critical steps in the SUMO cycle (Fig. 2). SUMO is synthesized in precursor form bearing a C-terminal extension that needs to be processed to expose a di-glycine motif. Analogous to the ubiquitin system, this function is carried out by dual function enzymes that are both precursor processing proteases and isopeptidases. The latter activity releases SUMO from conjugates allowing it to reenter the conjugation cycle.

Two ‘ubiquitin-like modifier proteases’, Ulp1 and Ulp2, were found in *S. cerevisiae* (Fig. 2). Ulp1 was selected from a gene pool for its ability to cleave a SUMO fusion protein in vitro [107]. Ulp1 is a cysteine protease whose active site appears to be structurally related to those of adenoviral processing proteases [107]. An essential function of Ulp1 is the C-terminal processing of SUMO precursor. Expression of mature SUMO in *ulp1* null mutants, however, enabled only extremely poor growth, indicating that deconjugating isopeptidase activity is another important property of this protease [107]. At nonpermissive conditions, conditional *ulp1* mutants display cell cycle defects very similar to conditional E1 and E2 mutants, consistent with defects in SUMO conjugation being the main cause of these defects [40,54,107]. Supporting this notion was the finding that ectopic expression of mature SUMO partially suppressed the temperature sensitive growth defect of an *ulp1-ts* mutant [40].

Ulp2/Smt4 was identified due to weak similarities of its sequence to Ulp1 [108] and because null mutations in the *ULP2* gene suppressed the temperature sensitivity of a *uba2* mutant defective in SUMO activation [40]. The latter observation and the fact that mutations in the SUMO conjugation machinery and *ulp2* mutants mutually suppressed each other demonstrated that SUMO conjugation and Ulp2-mediated deconjugation must be balanced for proper execution of SUMO function [40,108]. Ulp2 appears to be a desumoylating enzyme that has little, if any, in vivo SUMO precursor processing activity [40] although large amounts of *E. coli*-expressed Ulp2 displayed a weak processing activity in vitro [108]. Unlike *ULP1*, *ULP2* is not essential for viability, but *ulp2*-Δ mutants grow poorly, display defects in nuclear division and are sensitive to heat stress, DNA damage and microtubule destabilizing drugs.

Ulp1 and Ulp2 appear to have largely nonoverlapping functions. Deleting *ULP2* from *ulp1-ts* mutants did not result in a synthetic enhancement of the phenotype. Instead the two mutations mutually suppressed each other [40,108]. This result can again be rationalized by a

counterbalancing of *ulp1* mutants' defects in SUMO precursor processing, and thus in conjugation, by the *ulp2* deconjugating defect. A comparison of the SUMO conjugate pattern in *ulp1* and *ulp2* mutants suggests that the two deconjugating enzymes have different substrate specificity [40,108]. A clue as to how the difference of these two desumoylating activities with respect to specificity and function is brought about came from studies on their subcellular localization. Whereas Ulp2 displays a somewhat uneven intranuclear distribution, Ulp1 was detected in the nuclear periphery [40,108]. An experiment that employed the *nup133* NPC clustering mutant indicated that Ulp1 is associated with nuclear pores [40]. This finding was subsequently corroborated by studies that detected interaction of Ulp1 with nucleoporins and karyopherins. Two hybrid experiments suggested an interaction of Ulp1 with the NPC components Nup42 and Gle1, the latter of which could be confirmed by coimmunoprecipitation [109]. Another study, however, demonstrated that an interaction with these nucleoporins is not required for NPC association of Ulp1 [110]. Instead it was shown conclusively that Ulp1 forms a complex with the karyopherins Pse1, Kap95/importin β and Kap60, which is not dissociated by RanGTP in vitro [110]. Ulp1 appears to be tethered to the NPC by interaction of its N-terminal domain with these proteins. Experiments using certain truncated versions of Ulp1 suggested that the ~200-residue catalytic C-terminal domain of Ulp1 needs to be excluded from the nucleoplasm for viability. Interestingly, artificial targeting of the catalytic Ulp1 domain either to the cytoplasmic or nuclear face of the NPC both sustained *ulp1* viability, just as cytoplasmically localized Ulp1 did. Overexpression of the C-terminal domain of Ulp1 alone or fused to an NLS, in contrast, inhibited growth [34,110]. Overexpression of Ulp1, its partial mislocalization to the nucleoplasma or truncation of its N-terminal regulatory domain suppressed the growth defects of the *ulp2* mutant [108,110,111]. These data indicated that Ulp1 recognizes substrates of Ulp2 when present in the nucleoplasma. At the same time mislocalized Ulp1 appears to be less efficient towards the normal Ulp1 substrates implicating NPC localization in controlling its substrate specificity [111]. The observed toxicity of Ulp1 that is efficiently targeted to the cell nucleus could either be explained by higher activity of Ulp1 compared to Ulp2, or by a lack of an appropriate regulation of its activity. Ulp2, in contrast to Ulp1, appears to be modified in a cell cycle-dependent manner, which may provide such a regulation of its activity (our unpublished data). NPC localization of Ulp1 via karyopherins is consistent with a postulated function of this protease in desumoylating substrates that are passing through the nuclear pore (discussed in a later section).

A crystal structure of the C-terminal catalytic 'Ulp' domain in a complex with a covalently trapped SUMO has been reported. The structural analysis confirmed that Ulp-type proteases belong to a cysteine protease superfamily [34,107]. It shares with other Cys proteases similarities in its active site that include a catalytic triad (Cys-His-Asp). Ulp1, however, has several conserved structural motifs that are directly involved in SUMO binding, which distinguishes it from other Cys proteases including deubiquitylating enzymes [34]. The Ulp1 structure contains a narrow hydrophobic tunnel in close proximity to the active site providing recognition of the Gly-Gly-X motif at the C terminus of SUMO.

S. pombe Ulp1 (SpUlp1) combines properties described above for *S. cerevisiae* Ulp1 and Ulp2, as if it were an "all in one" enzyme. It has SUMO precursor processing activity as well as isopeptidase activity. Its localization is regulated by the cell cycle. During S and G2 phases, SpUlp1 localizes to the nuclear envelope, whereas it is observed within the nucleus during mitosis [112]. (Note that both yeast species exhibit a closed mitosis without breakdown of the nuclear envelope, and that a G1 phase is hardly detectable in *S. pombe*.)

Sequence analyses suggested the presence of seven Ulp genes in mammals, which encode proteins all sharing sequence similarity in their C-terminal domain with the catalytic 'Ulp' domain of Ulp1 [113]. At least one of these proteins (DEN1/NEDP1/SENP8) turned out to be a protease specific for NEDD8 [114,115]. The N-terminal domains of these proteases are very divergent, suggesting that these enzymes are functionally distinct. Thus far characterized as desumoylating enzymes are SMT3IP1/SENP3 which is localized in the nucleolus [116], SUSP1/SENP6 which is localized in the cytoplasm and especially abundant in tissues of reproductive organs [117], SENP1/SuPr-2 which is detected in the nucleus and accumulates in speckled foci [118,119], as well as SENP2/Axam, SMT3IP2/Axam2 and SuPr-1 which are isoforms apparently produced from the SENP2 gene by alternative splicing [120,121]. Axam and Axam2 have been implicated in the down-regulation of β-catenin although these were detected, respectively, either in the nucleus or the cytoplasm. Remarkably, desumoylation activity does not appear to be essential for their effect on β-catenin [120,122]. Similar to yeast Ulp1, SENP2/Axam is associated with nuclear pores [41]. It has been reported to be localized to the nucleoplasmic face of the NPC via interaction of its N-terminal domain with Nup153 [42]. Whether this interaction is sufficient for NPC localization of SENP2 has been disputed in the context of NPC tethering of yeast Ulp1 by karyopherins [110]. The shorter isoforms Axam2 and SuPr-1, which lack the N-terminal NPC targeting sequence, were detected in the cytosol and PML bodies, respectively. Upon overexpresssion, SuPr-1 has been shown to deconjugate SUMO from PML resulting in a disruption of PML bodies, which in turn results in the activation of c-jun-mediated transcription [121]. Enzymatic activity of SuPr-1, surprisingly, was not required for these effects, suggesting that binding to and sequestering of PML by SuPr-1 mimics the effects of desumoylation [121]. The available information on mammalian Ulp-type SUMO

cleaving enzymes suggests that their divergent substrate spectrum, similar to what was discussed above for yeast Ulp1 and Ulp2, is at least in part determined by differences in their subcellular localization.

An intriguing example of the utilization of an Ulp enzyme to deregulate the host cell SUMO system is provided by the human pathogen *Yersinia* [123]. Using a type III secretion system, this bacterium injects, among other proteins, a SUMO-deconjugating enzyme (YopJ) related to Ulp enzymes into to the host cells. YopJ injection results in an inhibition of the cells immune response by preventing activation of MAP kinase and NF-κB pathways. The underlying mechanism of this inhibition is still unclear. A related protease (XopD) is injected into cells of tomato or pepper plants by *Xanthomonas* species causing the so-called spot disease. Genetic data, however, indicate that XopD is not required for pathogenesis [124].

10. Substrates and functions of SUMO protein modification

Studies on the molecular biology of enzymes of the SUMO system summarized above, while pointing to a variety of functions of this protein modification in eukaryotic cell biology, did not reveal the secrets as to how sumoylation affects the function of proteins. Answers to this question depended and will depend on the identification of SUMO targets and experiments that probe for the function of their sumoylation. An increasing number of SUMO targets has been identified recently mainly due to the awareness of this modification and the consequent application of procedures that prevent rapid desumolyation upon cell lysis [125]. Many of these targets are mammalian proteins involved in signal transduction and transcriptional regulation. Others are involved in DNA damage repair, chromosome segregation and other processes (Table 1). What appears to emerge from the analysis of SUMO targets is that their modification alters their activities, their ability to interact with other proteins, or their subcellular localization. In vitro sumoylation system based upon purified proteins or *E. coli* extracts expressing a complete set of the enzymes may help to understand how the biochemical properties of a given substrate are changed by sumoylation [20,67,126].

11. Role of SUMO in cytosol/nucleus trafficking

As mentioned above, RanGAP was among the first proteins shown to be covalently modified by SUMO. Modified RanGAP is targeted to the cytoplasmic filaments of the NPC where it binds to RanBP2, which itself is a SUMO E3 and a binding site for Ran [14–16,18,20]. RanGAP activates the GTPase activity of Ran which results in the release of cargo from exportins that exit the nucleus in a complex with RanGTP. Released RanGDP can than engage in forming transport complexes with importins and their cargo, which are translocated into the nucleus. Thus, the Ran GTPase cycle is essential both for nuclear export and import (reviewed in Ref. [127]). In vitro data suggested that SUMO-dependent localization of RanGAP to the NPC is critical for nuclear import [15]. Yeast RanGAP (Rna1), however, appears not to be sumoylated and is largely cytosolic. An orthologue of RanBP2 has not been identified in *S. cerevisiae*. Genetic data, however, demonstrated that in this yeast a functional SUMO conjugation system is nonetheless required for import of proteins with so-called classical nuclear localization signals (NLS), whereas other tested import pathways and the export of mRNA were unaffected in the mutants studied [128]. More genetic evidence for an important role of SUMO protein modification in nuclear import came from studies in *Drosophila*. The *semushi/ubc9* mutant showed a defect in nuclear targeting of the segmentation transcription factor bicoid [129].

The observation that a SUMO ligase (RanBP2) as well as SUMO isopeptidases (Ulp1 and SENP2/Axam) are located at the NPC is consistent with a model, in which substrates are sumoylated and desumoylated upon their passage through the NPC [11,20]. In support of this hypothesis, it has been shown that the nuclear import of a number of proteins depends on their modification with SUMO [105,130–133]. There are, however, also examples of proteins whose export from the nucleus depends on their sumoylation [134,135] (for more detailed reviews on this subject, see Refs. [6,96,136]). In conclusion, there is accumulating evidence for a role of SUMO in various transport processes across the NPC. The molecular mechanisms, however, are still largely unclear.

12. Role of SUMO in protein targeting to subnuclear structures

In mammals, aside of modified RanGAP1, a large fraction of SUMO conjugates are associated with microscopically detectable subnuclear structures known as nuclear dots, PML nuclear bodies, promyelocytic oncogenic domains (POD), ND10 (nuclear domain 10) or Kr bodies [137,138]. The integrity of PML bodies appears to be important for normal cell growth and development, since their disruption is linked to human diseases such as acute promyelocytic leukemia (APL) and spinocerebellar ataxia type I (SCA1). Consistent with these findings is the observation that PML$^{-/-}$ mice are more susceptible to tumorigenesis and bacterial infections [139,140]. PML bodies have been proposed to be nuclear reservoirs for cellular regulators involved in the control of a variety of processes including stress response and apoptosis (reviewed in Ref. [141]). A number of viral proteins, some of which themselves are SUMO targets, interfere with the formation

of PML bodies [142–145] (reviewed in Ref. [146]). The large interferon-inducible antiviral GTPase Mx, interestingly, also localizes to PML bodies and interacts with components of the SUMO system [147].

Sumoylated proteins detected in PML bodies include promyelocytic leukemia (PML) protein, Sp100, CBP and Daxx, just to name a few [121,138,148–151]. Sumoylation of PML is required for the formation of these nuclear domains as they are absent from $Pml^{-/-}$ cells transfected with a PML gene carrying mutations eliminating the sumoylation sites [151,152]. Whereas some proteins such as Sp3 are mislocalized when their sumoylation sites are eliminated [153], the localization to PML bodies of most other proteins studied, including Sp100, Daxx, Topors, Lef1 and Hsf2, does not depend on their sumoylation [82,150,154–156]. An explanation for the latter results is that these proteins are targeted to these structures via interaction with other proteins. A hierarchy of interactions with PML as an essential component could account for the data [157].

SUMO modification, in addition, has been linked to other nuclear structures. Polycomb group (PcG) bodies have been proposed to be sumoylation centers based on the observation that PC2 mediates localization of SUMO and Ubc9 to these structures [101]. TEL bodies are transient intranuclear structures that are formed during S phase. They are characterized by the presence of SUMO-modified transcriptional repressor TEL/ETV6 [158]. A functional link between the transient formation of these structures and sumoylation of TEL remains to be established.

13. Role of SUMO in mitochondrial fission

The dynamin related protein DRP1 (mammalian orthologue of *S. cerevisiae* Dnm1), which is involved in mitochondrial fission, was recently reported to bind to Ubc9 and SUMO in a two-hybrid assay, and to be sumoylated in mammalian cells [159]. DRP1-SUMO appeared to be preferentially localized at the site of mitochondrial fission. Overexpression of SUMO-1, moreover, resulted in a stabilization of DRP1 and in an induction of mitochondrial fragmentation. Taken together, these data suggested the involvement of SUMO modification in the control of mitochondrial fission. In the same study it was reported that a large number of SUMO conjugates are to be found in mitochondrial protein preparations indicating that sumoylation is a common mitochondrial modification [159].

14. Role of SUMO in the regulation of transcription factors

An increasing number of reports have implicated sumoylation in the regulation of transcription factors (reviewed in detail in Refs. [10–13,93,94]). The potency to activate transcription of some activators appears to be enhanced, whereas many others are inhibited by sumoylation (see Table 1). Inhibition has often been correlated with redistribution of the transcriptional activators to nuclear bodies. By the same principle, the inhibition of transcriptional repressors has been linked to their sumoylation-dependent translocation to these nuclear reservoirs. In other cases sumoylation affects the interaction with co-repressors or co-activators. An example of such a mechanism by which an activator is turned into a repressor was observed for Elk-1. This transcriptional activator acts together with SRF in response to mitogenic stimulation. SUMO-modified Elk-1, however, recruits histone acetylase HDAC2 to promoters, which results in transcriptional repression [160]. SUMO modification of histone H4 similarly promotes recruitment of histone deactylase and heterochromatin protein 1 and thereby mediates silencing [161]. Sumoylation of HDAC1 and HDAC4, moreover, was shown to enhance their ability to repress transcription [162]. In summary, sumoylation is involved in the regulation of transcription by multiple mechanisms.

15. SUMO as an inhibitor of ubiquitin-mediated degradation

One distinct mode of action of SUMO, that of antagonizing ubiquitin-mediated degradation, was uncovered in studies on IκBα on mammalian cells [163]. This inhibitor binds to NF-κB and mediates its translocation to the cytoplasm and keeps it there. NF-κB is a transcriptional activator mediating inflammatory responses. In stimulated cells, IκBα is degraded in a ubiquitin-dependent manner allowing NF-κB to reenter the nucleus and to activate transcription of its target genes (for review see Ref. [164]). Desterro et al. [163] found a fraction of IκBα to be sumoylated. They also noted that this fraction, in contrast to unmodified IκBα, was resistant to TNFα-induced ubiquitin-dependent degradation. In addition, they could show that overexpression of SUMO-1 inhibited IκBα-dependent transcriptional activation. Upon closer inspection, they found residue Lys21 that is embedded in a sumoylation consensus sequence to be the major target of this modification. Interestingly, the same Lys residue is also the target for ubiquitylation providing a rationale for the abovementioned inhibition of proteolysis by sumoylation. Phosphorylation of residues Ser32 and Ser36 by IκB kinase appears to differentially regulate these modifications. While it is required for IκBα ubiquitylation, it apparently inhibits sumoylation. As only a small fraction of IκBα is sumoylated, the physiological significance of this modification is presently unclear [165]. A possible explanation is that sumoylation of newly synthesized IκBα is involved in shutting down the inflammatory response by preventing its degradation [13,132]. An analogous system would be the inhibition of ubiquitin-dependent degradation of CREB

(cAMP response element binding protein) by sumoylation after hypoxia [132]. Hypoxia results in a rapid phosphorylation-dependent degradation of CREB, which in turn triggers the induction of proinflammatory genes such as TNFα, leading to the activation of NF-κB. However, after prolonged treatment with hypoxia conditions, increased amounts of sumoylated CREB and IκBα were detected [132]. Furthermore, overexpression of SUMO-1 stabilized CREB in hypoxia suggesting a similar mechanism as described above for IκBα [132]. The same study demonstrated that SUMO-1 is transcriptionally induced in hypoxia. Based on these and other data, Comerford et al. [132] proposed that SUMO-1 modification may represent a general anti-inflammatory signal that provides an "off switch" to the inflammatory response by stabilizing certain regulators and by nuclear targeting of others.

16. SUMO and the cell cycle

Mutations in the SUMO conjugation system in *S. cerevisiae* have revealed the importance of this protein modification for normal execution of the cell cycle. Mutants deficient in SUMO conjugation accumulate at G2/M in the cell cycle with duplicated DNA content, short spindles, unseparated sister chromatids and undivided nuclei [25,40,107,166,167]. These cells are unable to degrade Pds1 and mitotic cyclins [25,167]. The underlying SUMO targets have not been identified up until now. These defects in cell cycle progression of sumoylation mutants were partially suppressed by mutations affecting the desumoylating enzyme Ulp2, indicating that the sumoylation state of the critical substrates is controlled both at the SUMO conjugation and deconjugation levels [40,107]. Cells lacking *ulp2* lose chromosomes, and therefore viability, at high frequency and are hypersensitive to microtubule destabilizing drugs, indicating that chromosome segregation is impaired [40,107]. The genes encoding SUMO/Smt3 and Ulp2/Smt4 (*s*uppressor of *M*if *t*wo) were originally identified as high copy suppressor of a mutation in the centromeric protein Mif2, also pointing to a role of sumoylation in kinetochore function [23]. An *ulp2* mutant was in addition isolated in a screen for hydroxyurea-sensitive mutants defective for the S phase checkpoint. Analysis of these mutants revealed that they were defective in maintaining cohesion near the centromeres of sister chromatids [168]. This defect and the temperature sensitivity of growth were suppressed by increased expression of *TOP2*. Topoisomerase 2 was found to be SUMO-modified and to be a substrate of Ulp2. Failure to desumoylate Top2 appears to underlie the precocious separation of chromatids observed in *ulp2* mutants. A mutant Top2 that lacks all sumoylation sites suppressed both the cohesion defect and the temperature sensitivity of *ulp2*. These data indicated that sumoylation of Top2 inhibits its ability to promote centromeric cohesion. Pds5 is another protein required to maintain chromatid cohesion. Recent work indicated that, as for Top2, sumoylation of Pds5 promotes dissolution of cohesion [71].

In mammalian cells, SUMO modification is also involved in processes controlling chromosome segregation. As described in an earlier section, a complex of RanGAP-SUMO and RanBP2 plays a role in the maturation of kinetochores and their attachment to microtubules [97,98]. In this context, it is worth noting that proteins from PML bodies interact with centromeres. Daxx for example interacts with CENP-C, the mammalian orthologue of Mif2, and is detected not only in PML bodies but also at interphase centromeres [169]. Work on the *Drosophila ubc9* mutant *lesswright* has implicated SUMO modification also in the disjunction of homologous chromosomes in meiosis I [170].

Ulp2 has been shown to play a role in mitotic targeting of condensins to rDNA. Ulp2 itself was found to be associated with rDNA chromatin by ChIP analysis, and *ULP2* overexpression suppressed the defects of a condensin mutant [171].

Many SUMO targets such as Top2, Pds5, PCNA and septins in yeast, as well as for example TOPII in mammalian cells, are sumoylated in a cell cycle-controlled manner indicating that SUMO modification serves to synchronize the function of many of its substrates with the cell cycle [29,71,125,168]. As not all substrates are modified during the same phase of the cell cycle, PCNA for example is modified in S phase whereas septins are sumoylated in G2/M [70,125], this raises the question as to how the modification of these substrates is regulated by the conjugation/deconjugation machinery.

17. SUMO in DNA damage repair and regulation of genomic stability

Genetic data obtained with yeast mutants defective in the SUMO cycle implicated SUMO modification in the DNA damage response. DNA damage hypersensitivity was observed in *S. pombe* cells lacking SUMO/Pmt3 and in *S. cerevisiae ubc9* and *ulp2* mutants [26,40,70,108]. These defects of *ulp2* mutants were attributed to their inability to recover from damage-induced checkpoint arrest [108].

The replication processivity factor PCNA (Pol30, "ring clamp") provides another link of SUMO to DNA repair processes. It was shown to be modified by the attachment of SUMO in the S phase of the cell cycle and upon exposure to lethal doses of methyl methanesulfonate [70]. Interestingly, the same lysine residue of PCNA that is the acceptor site for SUMO is also an attachment site for a Rad6/Ubc2-dependent ubiquitylation that occurs in response to nonlethal doses of DNA damaging reagents. Similar to what was previously shown for IκBα [163], therefore sumoylation of PCNA may antagonize ubiquitin-dependent processes. In the case of IκBα, as discussed earlier, sumoylation protects it from ubiquitin-mediated proteolysis. In the case of PCNA the effect of sumoylation appears to be different. Ubiquitylation

of PCNA appears not to target it for degradation but to activate it for participation in DNA repair processes mediated by the translesion polymerases η and ζ [70,172]. Spontaneous mutagenesis dependent on polymerase ζ can be stimulated either by sumoylated or mono-ubiquitylated PCNA. As sumoylation of a fraction of PCNA occurs in S phase [70], it was proposed that SUMO-modified PCNA may recruit polymerase ζ to overcome replication fork blocks not caused by induced DNA damage [172]. Another possibility is that sumoylation restricts PCNA to engage in DNA replication. Consistent with this assumption are genetic data showing that sumoylation of PCNA inhibits DNA repair and induced mutagenesis [172,173]. Genetic evidence suggested that sumoylation of PCNA on Lys164 inhibits Rad52-dependent recombinational repair, which may reduce the risk of chromosome rearrangements during S phase. The functional significance of the strong sumoylation on this residue observed upon high doses of DNA damaging reagents is unclear. Preventing this modification by changing Lys 164 to Arg increases the DNA damage sensitivity of cells that are unable to ubiquitylate this site [70]. A prevention of excessive chromosomal recombination by sumoylation of PCNA may also be a relevant mechanism under conditions that induce very high levels of DNA damage.

A recently discovered target implicated SUMO modification in another DNA repair process, base excision repair, in mammalian cells. Sumoylation of the human thymine-DNA glycosylase (TDG) on a single Lys residue facilitates its enzymatic turnover. [174]. Sumoylation of TDG is stimulated by DNA binding consistent with a model in which this modification triggers release of TDG from abasic sites following the hydrolysis of the mismatched base [174].

Other SUMO targets that implicate sumoylation in the DNA damage response are p53 and NEMO/IKKγ. DNA damage-induced sumoylation of the tumor suppressor p53 appears to increase its capacity for transcriptional activation of its target genes [175,176]. Genetoxic stress-induced activation of IκB Kinase (IKK) depends on sequential modification of its regulatory subunit NEMO with SUMO and ubiquitin [133]. Activated IKK phosphorylates IκB, which triggers its ubiquitin-mediated proteolysis thereby releasing transcription factor NF-κB. Sumoylation of NEMO induces its relocalization to the nucleus where it is desumoylated and ubiquitylated. The latter modification appears to depend on DNA damage-induced activity of the ATM (Ataxia telangiecstasia mutant) kinase. So modified NEMO then finally activates IKK in the cytosol [133].

18. Functions of the SUMO system in plants

A systematic analysis of genes of the SUMO conjugation machinery in *A. thaliania* based on the available genome sequence revealed a high degree of complexity of the system in this model plant [32]. Eight genes encoding SUMO isoforms, and 12 genes for putative Ulp-type deconjugating enzymes were detected.

A mutation affecting a SUMO deconjugating enzyme was identified in a screen for *Arabidopsis* mutants with abnormal flowering behavior [177]. The *esd4* (early in short days 4) mutant is characterized by an early flowering phenotype under short days conditions. The *ESD4* gene was found to encode a Ulp-type deconjugating enzyme capable of processing SUMO fusion proteins in vitro. However, it appears likely that not its processing function but rather the ability of Esd4 to deconjugate SUMO is required to control flowering, as overexpression of mature SUMO in *esd4* mutant plants enhanced the early flowering phenotype [177]. Moreover, *esd4* mutants contained reduced amounts of free SUMO but increased amount of SUMO–protein conjugates. As the relevant SUMO targets have not been identified, the role of sumoylation in this regulatory system remains obscure. Likely mechanisms include the premature transcriptional activation of the flowering program either by sequestering repressors or by enhancing the activity of transcriptional activators.

Other studies based upon overexpression of SUMO suggested roles of sumoylation in the modulation of the ABA signal transduction pathway and the induction of plant defence response [178,179].

19. SUMO and diseases

Recent studies have linked SUMO modification to important diseases. SUMO appears to influence the generation of amyloid β peptide (Aβ) from amyloid precursor protein by an unknown mechanism [180]. Increased protein sumoylation induced by overexpression of SUMO-3 inhibited the generation of Aβ. Overexpression of SUMO-3-K11R, in which the Lys residue involved in the formation of polySUMO chains is eliminated, had the opposite effect suggesting that monosumoylation enhances and polysumoylation inhibits Aβ formation [180].

Several studies have implicated SUMO modification also in the modulation of neurodegenerative diseases caused by polyglutamine (polyQ) repeat expansion such as Huntington's disease (HD), spinocerebellar ataxias (SCA), Machado–Joseph disease or spinal and bulbar muscular atrophy (SBMA) [165,181,182]. Neurons in affected brain regions of patients showed increased SUMO-1 staining, and transgenic mice expressing mutant ataxin-1 exhibited accumulation of sumoylated proteins in the cerebellar cortex [181,183]. Using a *Drosophila* model of HD, it was demonstrated that a fragment of Huntingtin (Htt) with an expanded polyQ tract is modified both by sumoylation and by ubiquitylation [165]. While ubiquitylation of Htt abrogated neurodegeneration, sumoylation exacerbated it. Sumoylated Htt is stabilized, is less likely to aggregate, and has an increased capacity to repress transcription. It was suggested that sumoylation increases the accumulation of

toxic soluble Htt oligomers, which mediate neurodegeneration via transcriptional repression [165].

Neuronal intranuclear inclusion disease is another neurodegenerative disorder that is characterized by nuclear inclusions that are similar to those found in polyQ expansion diseases [184]. These nuclear inclusions, which are detectable as 10-nm fibrils in electron microscopy, were immunopositive for SUMO-1 indicating that SUMO-modified proteins are recruited to these insoluble structures.

Most recent reports in this context concern SUMO-4. A polymorphism (SNP) leading to an exchange of a conserved Met residue at position 55 to Val (SUMO-4M versus SUMO-4V) has been detected in the human population. SUMO-4M expression has been shown to result in an increased expression of heat shock promoters. A weak familial association with type I diabetes mellitus was detected for SUMO-4M [30]. Based on these findings it was proposed that SUMO-4(M) modification of HSF1 and/or HSF2 might promote type I diabetes [30].

20. Concluding remarks

Since the discovery of protein modifier SUMO about 8 years ago, we have learned much about the enzymatic system mediating SUMO conjugation and deconjugation. It is unclear, however, how many enzymes of this system, in particular of the substrate specificity-mediating SUMO ligase class, are yet to be discovered. The number of proteins identified as substrates of SUMO modification is rapidly increasing and, due to proteomic approaches, is likely to reach a peak soon. While the growing list of substrates gives an impression of the pleitropic nature of the functions of SUMO modification in a wide range of cellular processes, much remains to be understood about how sumoylation modifies the functions of these proteins. Is there a unifying principle or are there multiple or even many modes of actions of SUMO? Detailed functional studies on many SUMO substrates will be required to answer this question. A few insightful examples point to one mode in which sumoylation is antagonistic to ubiquitylation for some substrates, and to another mode in which sumoylation is either required for protein interaction (e.g., interaction of RanGAP-SUMO with RanBP2) or inhibitory to it. The latter appears to be a recurring mechanism in the down-regulation of transcription factors. An increasing body of evidence implicates sumoylation in targeting of certain proteins to the cell nucleus and to subnuclear structures. The underlying mechanisms, however, are still largely unclear.

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