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# PROTEIN MODIFICATION BY SUMO

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**Key Words** post-translational modification, ubiquitin-like protein, PIAS, Ubc9, Ulp

■ **Abstract** Small ubiquitin-related modifier (SUMO) family proteins function by becoming covalently attached to other proteins as post-translational modifications. SUMO modifies many proteins that participate in diverse cellular processes, including transcriptional regulation, nuclear transport, maintenance of genome integrity, and signal transduction. Reversible attachment of SUMO is controlled by an enzyme pathway that is analogous to the ubiquitin pathway. The functional consequences of SUMO attachment vary greatly from substrate to substrate, and in many cases are not understood at the molecular level. Frequently SUMO alters interactions of substrates with other proteins or with DNA, but SUMO can also act by blocking ubiquitin attachment sites. An unusual feature of SUMO modification is that, for most substrates, only a small fraction of the substrate is sumoylated at any given time. This review discusses our current understanding of how SUMO conjugation is controlled, as well as the roles of SUMO in a number of biological processes.

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## INTRODUCTION

Covalent modifications of proteins are rapid, energetically inexpensive mechanisms for reversibly altering protein function, and modifications such as phosphorylation, acetylation, and ubiquitylation participate in most cellular activities. Ubiquitylation, which involves attachment of the 76-residue protein ubiquitin (Ub) to other proteins, often targets the substrate protein for degradation by the proteasome, but it can also have several other functions (1, 2). Recently, several small ubiquitin-like proteins (Ubls) that also act as post-translational modifications on other proteins have been discovered. These Ubls vary widely in their degree of sequence similarity to Ub but share a common chemistry for becoming attached to internal lysine residues in substrate proteins (3). Ubls have a variety of different functions, but they do not target their substrates directly for proteasome-dependent proteolysis. The Ubls with the widest range of functions and the most known substrates are the members of the SUMO (small ubiquitin-related modifier) family. Several previous reviews on SUMO cover earlier work and specific topics in depth (4–8).

SUMOs constitute a highly conserved protein family found in all eukaryotes and are required for viability of most eukaryotic cells, including budding yeast, nematodes, fruit flies, and vertebrate cells in culture (9–13). In multicellular organisms, SUMO conjugation takes place in all tissues at all developmental stages (14–21). Since its discovery in 1996, SUMO has been found covalently attached to more than 50 proteins, which include the androgen receptor, I$\kappa$B$\alpha$, c-jun, histone deacetylases (HDACs), p53, and other proteins that participate in transcription, DNA repair, nuclear transport, signal transduction, and the cell cycle. Most SUMO-modified proteins that have been characterized in mammalian systems are involved in transcription, which is often repressed by SUMO conjugation. However, genetic studies in model organisms have pointed to a role for SUMO in chromosome dynamics and higher order chromatin structures, illustrating the diversity of SUMO function.

At this time, only one fairly uninformative generalization about the downstream consequences of SUMO attachment is possible: SUMO alters substrate interactions with other macromolecules. SUMO often has a positive effect on protein-protein interactions, and it promotes assembly of several multi-protein complexes. However, the effects of SUMO on interactions vary for different substrates. For example, sumoylation allows RanGAP1 to bind tightly to the nuclear pore complex protein RanBP2/Nup358 (22, 23), but no other sumoylated

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proteins participate in a stable complex with RanBP2. SUMO can also act by a completely different mechanism: preventing ubiquitylation of a protein by blocking the lysine where Ub would normally be attached (24–27).

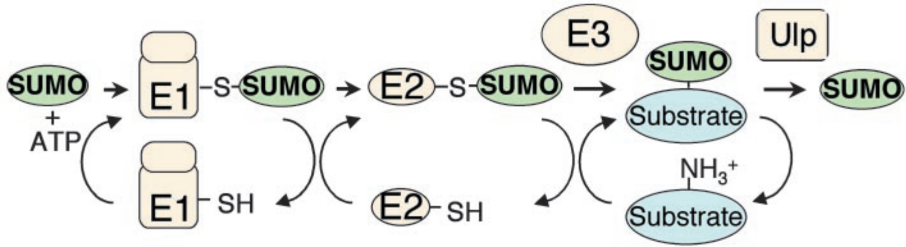
There are several reasons why proteins that have been intensely studied for many years, such as c-jun and the androgen receptor, have only recently been shown to be modified by SUMO. One is that SUMO-cleaving enzymes rapidly desumoylate all conjugates instantly upon cell lysis, unless cells are lysed under denaturing conditions or cleaving enzymes are inhibited. Another is that usually only a small fraction of the substrate, often less than 1%, is sumoylated at any given time. A third reason for the late discovery of SUMO is that, for some sumoylated proteins, eliminating the SUMO attachment site has fairly subtle effects on protein function, so that functional domains containing the attachment sites were not immediately apparent.

However, recent experiments have uncovered a variety of effects that can clearly be attributed to sumoylation of specific proteins at specific sites, and new substrates and functions for SUMO continue to be discovered at a rapid pace.

## THE SUMO CONJUGATION PATHWAY

The linkage between SUMO and its substrates is an isopeptide bond between the C-terminal carboxyl group of SUMO and the $\epsilon$-amino group of a lysine residue in the substrate. A three-step enzyme pathway attaches SUMO to specific substrates, and other enzymes cleave SUMO off its targets (Figure 1). The enzymes of the SUMO pathway, although analogous to those of the Ub pathway, are specific for SUMO and have no role in conjugating Ub or any of the other Ubls.

The SUMO pathway begins with a SUMO-activating enzyme (also called an E1), which carries out an ATP-dependent activation of the SUMO C terminus and then transfers activated SUMO to a SUMO-conjugating enzyme (E2) called Ubc9. SUMO is then transferred from Ubc9 to the substrate with the assistance of one of several SUMO-protein ligases (E3s). Ubc9 and the E3s both contribute to substrate specificity. Many of the Lys residues where SUMO becomes attached are in the short consensus sequence ΨKXE, where Ψ is a large hydrophobic amino acid, generally isoleucine, leucine, or valine; K is the lysine residue that is modified; X is any residue; and E is a glutamic acid. This motif is bound directly by Ubc9. E3s probably enhance specificity by interacting with other features of the substrate. Sumoylation is a reversible modification, and removal of SUMO is carried out by enzymes of the Ulp family that specifically cleave at the C terminus of SUMO. Ulps are also required for generating mature SUMO from the SUMO precursor, which contains a short peptide blocking its C terminus.




| | E1 | E2 | E3 | SUMO-cleaving protease |
|---|---|---|---|---|
| *S. c.* | Aos1·Uba2 | Ubc9 | Siz1<br>Siz2 (Nfi1) | Ulp1<br>Ulp2 (Smt4) |
| *H. s.,*<br>*M. m.,*<br>*R. n.* | Aos1·Uba2<br>(SAE1,Sua1·SAE2) | Ubc9 | PIAS1 (GuBP)<br>PIAS3 (KChaP)<br>PIASxαβ (ARIP3,Miz1)<br>PIASy<br>RanBP2 (Nup358)<br>Pc2 | SENP1<br>SENP2 (Axam,SuPr-1,SMT3IP2)<br>SENP3 (SMT3IP1)<br>SENP6 (SUSP1) |

**Figure 1** The SUMO conjugation pathway. (*top*) Enzymes and reactions of the SUMO pathway are described in the text. (*bottom*) Enzymes present in *S. cerevisiae* (*S.c.*) and in human (*H.s.*), mouse (*M.m.*), and rat (*R.n.*) are listed. Alternative names and names of splice variants are in parentheses.

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## SUMO

SUMOs share only ~18% sequence identity with Ub, but the folded structure of the SUMO C-terminal Ub-like domain is virtually superimposable on that of Ub (28) (Figure 2). However, the surface charge topology of SUMO is very different from that of Ub, with distinct positive and negative regions (28). SUMOs are ~11 kDa proteins, but they appear larger on SDS-PAGE and add ~20 kDa to the apparent molecular weight of most substrates. SUMOs are ~20 amino acids longer than Ub, and the extra residues are found in an N-terminal extension, which is flexible in solution. The N-terminal extension of yeast SUMO can be entirely deleted with only modest effects on SUMO function, indicating that the Ub-like domain is sufficient for conjugation to many substrates and for any downstream interactions required for yeast viability (29). All SUMO genes actually encode a precursor bearing a short C-terminal peptide, which is cleaved off by Ulps to produce the mature Gly-Gly C terminus found in most Ubls.

The yeast and invertebrates studied to date contain a single SUMO gene, whereas vertebrates contain three: SUMO-1 (also known as sentrin, PIC1, GMP1, Ubl1, and Smt3c), SUMO-2 (sentrin-3, Smt3a), and SUMO-3 (sentrin-2, Smt3b) (13, 15, 16, 22, 23, 30–33). Plants contain even more SUMO genes, with

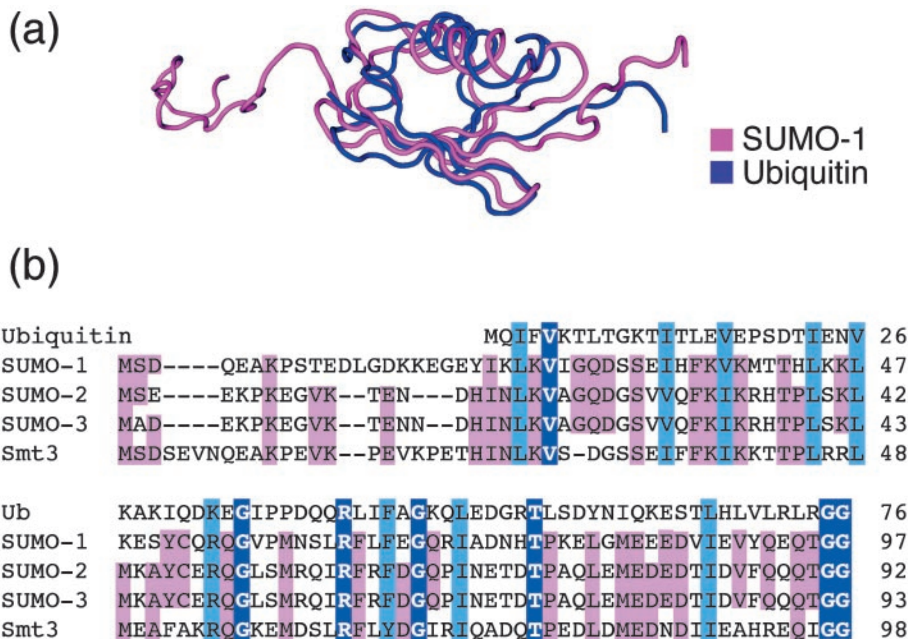


**Figure 2** Comparison of SUMO and ubiquitin. (*a*) Structural alignment of the backbones of SUMO-1 (*pink*) and ubiquitin (*blue*) is from the VAST database (NCBI) with structures from References 28 and 28a. The N termini are on the left and and the C termini on the right. The SUMO structure is of the precursor and includes the C-terminal tetrapeptide that is cleaved off. (*b*) Sequence alignment of *H. sapiens* Ub, SUMO-1, SUMO-2, and SUMO-3 and the *S. cerevisiae* SUMO protein Smt3 was made using ClustalW. Positions that are identical in all sequences are shaded dark blue, and conserved positions are light blue. Positions that are identical in at least three of the SUMO proteins, but not in Ub, are shaded pink.

eight in *Arabidopsis* (20, 21). The single SUMO genes in the nematode *Caenorhabditis elegans* and the budding yeast *Saccharomyces cerevisiae* are essential for viability, while fission yeast *Schizosaccharomyces pombe* lacking the SUMO gene *pmt3* are barely viable and have severe defects in genome maintenance (13, 32, 33).

Mammalian SUMO-2 and -3 share ~95% sequence identity with each other and are ~50% identical to SUMO-1. Although the same E1 and E2 enzymes activate and conjugate all SUMO isoforms, SUMO-1 appears to have a partially distinct function from SUMO-2 and -3, which are assumed, at present, to be functionally identical. Cells contain a large pool of free, unconjugated SUMO-2/3, but there is virtually no pool of free SUMO-1; at any given time, the vast majority of SUMO-1 is conjugated to other proteins (23, 34). Furthermore, conjugation of SUMO-2/3 is strongly induced in response to various stresses, but

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SUMO-1 conjugation is not (34). Plants have a similar pattern of SUMO isoform utilization, with some isoforms conjugated primarily under stress conditions (20, 21). Thus, one function of SUMO-2/3 may be to provide a reservoir of free SUMO for stress responses. There is also evidence that different SUMOs are used preferentially for different substrates. RanGAP1 is the major substrate of SUMO-1, but it is not strongly modified by SUMO-2/3 (34). Other proteins can be modified equally well by SUMO-1 and SUMO-2/3 (35, 36). It is likely that E3s mediate the differential conjugation of the SUMO isoforms (see below).

Another difference between SUMO-1 and SUMO-2/3 is that SUMO-2 and -3 contain ΨKXE sequences in their N-terminal extensions, which can serve as SUMO attachment sites, thereby allowing formation of poly-SUMO chains (37). Yeast SUMO also contains a ΨKXE sequence and can form chains (29, 38, 39). Chain formation by SUMO was a surprise because in vivo most SUMO attachment-site Lys residues bear only a single copy of SUMO, although proteins are often multiply sumoylated by attachment of mono-SUMO at different sites (40, 41). The only protein on which a SUMO-2 chain has been observed in cells is the histone deacetylase HDAC4; it forms a di-sumoylated conjugate that disappears when the SUMO attachment site in SUMO-2 is mutated (37). However, there are intriguing data suggesting that cleavage of the amyloid precursor protein to generate the amyloid $\beta$ peptide involves SUMO-2/3 chain formation (42). The function of SUMO chains is unclear in yeast, where chain formation can be eliminated without notable effects on either SUMO function or the pattern of conjugates (29, 43).

## SUMO-Activating Enzyme (E1)

Like the E1 for Ub, the SUMO-activating enzyme (E1) catalyzes a three-part reaction. First, the C-terminal carboxyl group of SUMO attacks ATP, forming a SUMO C-terminal adenylate and releasing pyrophosphate. Next, the thiol group of the active site cysteine in the E1 attacks the SUMO adenylate, releasing AMP and forming a high-energy thiolester bond between the E1 and the C terminus of SUMO. Finally, the activated SUMO is transferred to a cysteine in the E2. The crystal structure of the related E1 for the Ubl Nedd-8 suggests that three distinct domains catalyze each of the steps (44). Most organisms contain a single SUMO-activating enzyme, which is required for conjugation of all SUMO variants to all substrates. Interestingly, the SUMO E1 is a heterodimer, whereas the Ub E1 is a monomer, but both components of the SUMO enzyme are related to the Ub enzyme. Aos1 (also called SAE1, Sua1) resembles the N terminus of the Ub E1, while Uba2 (SAE2) corresponds to the C terminus and contains the active site cysteine (33, 45, 46). Although the two-subunit structure of the SUMO E1 suggests that Aos1 and Uba2 might function or be regulated separately, all cellular Uba2 and Aos1 is found in the heterodimer (47). However, *Arabidopsis* actually has two *SAE1* (*AOS1*) genes, whose products presumably each partner with the product of the single *SAE2* (*UBA2*) gene (21).

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## SUMO-Conjugating Enzyme (E2)

In the second step of the pathway, SUMO is transferred from the E1 to the active site cysteine of the SUMO-conjugating enzyme (E2), forming a SUMO-E2 thiolester intermediate. This serves as the SUMO donor in the final reaction in which SUMO is transferred to the amino group of a Lys in the substrate. Ubc9 is the only SUMO-conjugating enzyme in yeast and invertebrates and most likely in vertebrates as well (10, 13, 48, 49). The presence of only one SUMO E2 contrasts with the Ub pathway where multiple E2s participate in ubiquitylating distinct sets of substrates. Ubc9 shares considerable sequence similarity with ubiquitylation E2s and also assumes essentially the same folded structure, although Ubc9 has a strong overall positive charge (50). A patch surrounding the active site cysteine of Ubc9 binds directly to the ΨKXE consensus sequence in the substrate (51, 52). A second region on Ubc9, separate from the active site, binds directly to SUMO and is involved in transfer of SUMO from the E1 (39, 53). Like the genes for SUMO, Aos1, and Uba2, the gene encoding Ubc9 is essential in all organisms tested except *S. pombe*, in which the mutant lacking the Ubc9 gene *hus5* has the same phenotypes as mutants lacking SUMO, Aos1 or Uba2 (11–13, 32, 54–56).

## SUMO Ligases (E3s)

Three distinct types of SUMO ligases (E3s) have been discovered recently. One includes members of the PIAS (protein inhibitor of activated STAT) family (57, 58), originally discovered as inhibitors of STAT transcription factors (59); another consists of a domain in the large vertebrate nuclear pore protein RanBP2/Nup358 (60); and the third is the polycomb group protein Pc2 (61). These proteins meet the definition of an E3 in that they (*a*) bind the E2, (*b*) bind the substrate, and (*c*) promote transfer of SUMO from the E2 to the substrate in vitro (1). These SUMO E3s, like the RING domain-containing E3s involved in ubiquitylation, do not form covalent intermediates with SUMO, but instead they appear to act by bringing together Ubc9 and the substrate. They may also activate Ubc9. There was initially some doubt as to whether there would be E3s in the SUMO pathway because SUMO conjugation can take place in vitro in the absence of an E3, and this reaction is specific for the Lys residues that are actually modified in vivo (45, 46). However, the vast majority of sumoylation in yeast is E3-dependent (38, 62), and E3s enhance SUMO attachment in vitro to all substrates that have been tested (38, 60, 63–68). Together these results indicate that E3s participate in at least most of the sumoylation that occurs in cells.

PIAS FAMILY E3s PIAS proteins share a conserved ~400 residue N-terminal domain that includes several shorter regions of greater similarity, notably a SAP domain (SAR, Acinus, PIAS), which has been implicated in binding AT-rich DNA sequences (64, 69–71), and an SP-RING, which resembles the RING domains found in many ubiquitylation E3s (57, 58). Like RING domains, which

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bind ubiquitylation E2s, the SP-RING binds directly to Ubc9 and is required for the E3 activity of PIAS proteins, suggesting that it is the critical element for promoting the sumoylation reaction (63–65). PIAS proteins also contain a short motif of hydrophobic amino acids followed by acidic amino acids, called an SXS domain or SIM (SUMO interaction motif), which has been implicated in binding directly to SUMO (72). Deletion of the SIM has little effect on the ability of PIAS proteins to promote SUMO conjugation, but it can affect their localization and transcriptional effects (64, 66). The main differences between PIAS proteins lie in their 100–450 residue C-terminal tails, which share no sequence similarity with each other or with other known proteins. Some PIAS proteins also have splice variants that produce alternative C-terminal tails. It is likely that these C-terminal domains interact with specific substrates.

*S. cerevisiae* contains two PIAS family proteins, Siz1 and Siz2/Nfi1. Siz1 is required for sumoylation of septin family cytoskeletal proteins and of the replication processivity factor PCNA; whereas Siz2 does not promote septin or PCNA sumoylation but sumoylates other, as yet unidentified, proteins (24, 38, 62). Together, *SIZ1* and *SIZ2* are required for most sumoylation in yeast, but the *siz1*Δ *siz2*Δ double mutant still carries out low levels of SUMO conjugation. This double mutant is also viable, indicating that Siz-independent sumoylation can fulfill the essential functions of SUMO. However, the *siz1*Δ *siz2*Δ mutant does have significant growth defects not seen in either single mutant, suggesting that Siz1 and Siz2 have some overlapping functions. *Drosophila melanogaster* has a single PIAS gene, known as *dpias*, *Su(var)2–10,* or *zimp*, which produces at least two isoforms derived from alternative splicing. *dpias* is an essential gene that functions in chromosome organization and segregation as well as in blood cell and eye development (73–75).

Four mammalian genes encoding PIAS proteins have been described, *PIAS1* (also called *GuBP*), *PIAS3*, *PIASx*, and *PIASy* (59, 76, 77). PIAS3 has a splice variant called KChaP, and *PIASx* also produces two isoforms derived from alternative splicing, designated PIASx$\alpha$ (ARIP3) and PIASx$\beta$ (Miz1) (78–80). PIAS1 and PIAS3 are found in all cell types, whereas PIASx and PIASy appear to be expressed primarily in testis (76, 81). PIASx$\alpha$, PIASx$\beta$, PIASy, PIAS1, and PIAS3 all localize to intranuclear dots, which are, at least in part, PML nuclear bodies (see below) (64, 66, 82, 83).

By analogy with the Ub system, the purpose of the different PIAS proteins may be to sumoylate different substrates, but currently the only clear example of this is the specificity of Siz1 for septins and PCNA. Sumoylation of many vertebrate-derived substrates can be stimulated by several different PIAS proteins, upon overexpression both in cells and in vitro. For example, PIAS1, PIAS3, and PIASy can all promote sumoylation of p53 (63, 68). Such a result may suggest either that PIAS proteins have overlapping substrate specificities or that in vitro assays do not faithfully reproduce physiological substrate selection mechanisms. In support of this second possibility, Siz2/Nfi1 can stimulate SUMO attachment to septins in vitro, even though it is incapable of promoting

septin sumoylation in vivo (43). However, PIAS proteins do show different substrate specificities with some substrates: PIAS1 and PIASx$\beta$, but not PIASx$\alpha$, stimulate sumoylation of Mdm2 (82).

Another function of the different PIAS proteins may be to promote attachment of the different SUMO isoforms. PIASy preferentially conjugates SUMO-2, rather than SUMO-1, to the transcription factors LEF1 and GATA-2, and it strongly enhances overall SUMO-2 conjugation (64, 84). It is also not clear that all PIAS effects are mediated by SUMO conjugation. In particular, PIAS proteins inhibit binding of STAT transcription factors to DNA in vitro, and there is no evidence that this effect involves SUMO (76, 77, 85, 86).

RanBP2/Nup358 A second type of SUMO E3 consists of an ~300 residue region in the large vertebrate-specific nuclear pore protein RanBP2 (also called Nup358), which localizes to the cytoplasmic fibrils of the nuclear pore and contains several types of functional domains (60, 87, 88). The E3 domain, called the internal repeat (IR) domain, contains two repeats of an ~50 residue sequence that shares no sequence similarity with any of the known ubiquitylation E3s or any other protein. In addition to having the capacity to act as an E3 in the sumoylation of several proteins, including RanGAP1, the IR domain forms a stable trimeric complex with SUMO-RanGAP1 and Ubc9, and thus it is responsible for the localization of SUMO-RanGAP1 to the nuclear pore (89, 90). RanBP2 itself can also be sumoylated (60, 91). Presumably, sumoylation of nuclear proteins by RanBP2 would have to occur during nuclear import.

Although it has not been demonstrated conclusively that RanBP2 is required in vivo for sumoylation of proteins other than RanGAP1, in vitro results indicate that RanBP2 and PIAS proteins have mostly distinct sets of substrates, suggesting they may have fundamentally different specificities. The IR domain promotes SUMO attachment in vitro to several proteins, including HDAC4, Sp100, and RanGAP1, whose sumoylation is not stimulated by PIAS proteins. Conversely, PIAS proteins, but not RanBP2, stimulate sumoylation of p53 and Sp3 (60, 67, 92). However, other proteins can be sumoylated by either RanBP2 or PIAS proteins (82, 93).

Pc2 A third reported E3 for SUMO is the polycomb group (PcG) protein Pc2 (61). PcG proteins form large multimeric complexes that have histone methylation activity and that participate in transcriptional repression through establishment of epigenetically inherited domains of silent chromatin. The transcriptional corepressor CtBP associates with PcG bodies via Pc2, and Pc2 stimulates sumoylation of CtBP both in vivo and in vitro. Moreover, overexpression of Pc2 in cells causes SUMO and Ubc9 to colocalize at PcG bodies, suggesting that PcG bodies may be major sites of sumoylation. However, the enhancement of CtBP sumoylation by Pc2 in vitro is very modest (61), and PIAS1, PIASx$\beta$, and RanBP2 can also promote CtBP sumoylation (93), suggesting that there may be multiple factors involved in CtBP sumoylation.

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## SUMO-Cleaving Enzymes

The pattern of SUMO conjugates is dynamic and changes during the cell cycle and in response to various stimuli (94). SUMO-cleaving enzymes (also called isopeptidases) have at least two functions in this process: They remove SUMO from proteins, making the modification reversible, and they also provide a source of free SUMO to be used for conjugation to other proteins. Free SUMO is generated both from newly synthesized SUMO, which must be cleaved to remove a short C-terminal peptide, and from desumoylation of existing conjugates. Both of these sources of free SUMO are likely to be critical for maintaining normal levels of SUMO conjugation because cellular pools of unconjugated SUMO-1 and yeast SUMO are very low (23, 33).

All known SUMO-cleaving enzymes contain an ~200 amino acid C-terminal domain (the Ulp domain), which has the SUMO cleaving activity (95). The Ulp domain does not share sequence similarity with the enzymes that cleave Ub. Instead, it is distantly related to a number of viral proteases (94, 96). The different SUMO-cleaving enzymes have varying N-terminal domains, which are apparently regulatory and target the enzymes to different parts of the cell (97–100). Overexpression of the SUMO cleaving domain of the yeast enzyme Ulp1 is lethal in yeast, consistent with the likelihood that uncontrolled desumoylation is toxic (95).

Two desumoylating enzymes with distinct functions have been described in *S. cerevisiae*. Ulp1 localizes to the nuclear pore complex (NPC) and is required for cleaving both the SUMO precursor and SUMO conjugates to other proteins; whereas Ulp2/Smt4 localizes to the nucleus, does not cleave the precursor, and appears to desumoylate a distinct set of conjugates (94, 98, 101–103). Ulp1 and Ulp2 cannot compensate for each other functionally, as *ulp1*Δ cells are inviable, and *ulp2*Δ cells are stress sensitive and have defects in genome maintenance. The substrate specificity of Ulp1 is controlled by its N-terminal regulatory domain, which targets it to the NPC. Mutants lacking this domain both nonspecifically desumoylate Ulp2 targets and fail to desumoylate the normal targets of Ulp1 (97).

Seven genes in mammalian genomes encode proteins with Ulp domains, but at least one of these cleaves the Ubl Nedd-8 instead of SUMO (104–106). All have divergent N-terminal domains, and those that have been characterized localize to different parts of the cell, suggesting that they may desumoylate different proteins. These enzymes include SENP3 (SMT3IP1), which localizes to the nucleolus (107); SENP6 (SUSP1), found primarily in the cytoplasm (108); SENP1, which localizes to foci in the nucleus and the nuclear rim (109); and SENP2 (Axam, SMT3IP2/Axam2, SuPr-1), which produces at least three different isoforms derived from alternatively spliced mRNAs (110–112). Of these, the SENP2/Axam isoform has an N-terminal extension that allows it to bind the nucleoplasmic side of the nuclear pore complex (99, 100); Axam2/SMT3IP2 has a different N terminus and localizes to the cytoplasm

(112); and SuPr1 lacks these N-terminal domains and localizes to PML nuclear bodies (110).

## Substrate Specificity in Sumoylation

SUMO is attached to most substrates at the lysine in a ΨKXE sequence, but there are clearly other determinants involved in substrate selection as well. Of the positions in the consensus sequence, the glutamic acid is the most highly conserved position other than the lysine. In some cases, even a conservative Glu to Asp mutation significantly reduces sumoylation (92, 113), although a few ΨKXD sequences are sumoylated (40). The ΨKXE motif is bound directly by the E2 Ubc9 (114), and this direct interaction explains why so many sumoylation substrates have been identified via their interaction with Ubc9 in the yeast two-hybrid screen and also why the E1 and Ubc9 alone are sufficient to sumoylate many substrates at the correct sites in vitro in the absence of an E3. Remarkably, a ΨKXE sequence and a nuclear localization sequence (NLS) are sufficient to target an artificial substrate for sumoylation, indicating that the requirements for SUMO conjugation can be very simple (113). Most SUMO substrates localize to the nucleus, and many, including Sp100, HDAC4, Mdm2, and Smad4, require their NLSs for sumoylation (26, 67, 82, 115).

The ΨKXE motif is very short and is found in many proteins, most of which are probably not modified by SUMO. For example, out of 5884 open reading frames (ORFs) in *S. cerevisiae*, there are 2799 sequences of the form (IVL)KXE distributed in 1913 different ORFs. Thus, interactions other than those between Ubc9 and the ΨKXE motif are likely to be critical in determining which proteins are sumoylated. Most of these probably involve interactions between an E3 and the substrate or a substrate-associated protein. However, the crystal structure of the RanGAP1-Ubc9 complex shows an additional contact besides the ΨKXE interaction (51), suggesting that other interactions between the substrate and Ubc9 may also participate in substrate selection.

Several proteins are also modified at sites other than ΨKXE. The replication processivity factor PCNA has two sumoylation sites, one conforming to the consensus sequence and the other at a T*K*ET sequence (24). TEL, PML, Smad4, and the Epstein Barr virus BZLF1 protein have reported sumoylation sites at T*K*ED, A*K*CP, V*K*YC, and V*K*FT, respectively, and both lysines in a G*K*VE*K*VD sequence in Axin are sumoylated (116–120). Moreover, some sumoylated proteins, such as Mdm2, Daxx, CREB, and CTBP-2, do not contain a ΨKXE sequence; others are still sumoylated when all consensus sites are mutated (61, 82, 121–124). It is not known how these nonconsensus sites are recognized.

## Regulation of SUMO Conjugation

The set of proteins that is modified by SUMO changes during the cell cycle and in response to various conditions, but how SUMO conjugation is regulated is not

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well understood. In theory, sumoylation could be regulated at the level of either attachment or removal of SUMO; a change in either rate would alter the steady-state amount of protein modified. Some examples of proteins showing regulated SUMO modification are the yeast bud neck-associated septin proteins, which are modified only during mitosis and only on the mother-cell side of the bud neck (40). Septin sumoylation requires the E3 Siz1, which itself localizes to the mother-cell side of the bud neck exclusively during mitosis (38, 62). Thus, it is likely that septin sumoylation is regulated by controlling the localization of Siz1, possibly via phosphorylation of Siz1.

Phosphorylation of several substrates affects their sumoylation, mostly negatively. Phosphorylation of c-jun, PML, and I$\kappa$B$\alpha$ correlates with reduced SUMO attachment (25, 125, 126). Furthermore, the antagonistic relationship between phosphorylation and sumoylation is involved in activation of the transcription factor Elk-1 by MAP kinases (127). In unstimulated cells, sumoylated Elk-1 represses Elk-1-dependent gene expression (see below). Upon MAPK-dependent phosphorylation, Elk-1 is desumoylated and transcription is activated. However, phosphorylation has the opposite effect on sumoylation of the heat shock transcription factor HSF1, which must be phosphorylated in order to be sumoylated (128, 129).

Because lysines serve as the attachment sites for several modifications, which include Ub, other Ubls, acetylation, and methylation, it is possible that these modifications might regulate each other by competing for the same lysines. In fact, several proteins contain a lysine that can be modified by either Ub or SUMO (see below), and the transcription factor Sp3 contains a lysine that can be either acetylated or sumoylated (92, 130, 131).

Sumoylation of some proteins is regulated by binding interactions with other macromolecules. Sumoylation of Mdm2 and p53 in vivo is enhanced by association with the tumor suppressor ARF (132). In another example, sumoylation of the base excision repair protein thymine DNA glycosylase (TDG) in vitro is stimulated both by DNA and by the downstream enzyme in the repair pathway (36). In contrast, the transcription factor Sp3 is resistant to sumoylation when bound to DNA (92).

## BIOLOGICAL FUNCTIONS OF SUMO

Although identification of the enzymes of the SUMO pathway has proceeded rapidly, investigations of how SUMO affects biological processes are only at the early stages. Several features of the SUMO system, including the low levels of modification, the presence of Ulp activity in native lysates, and a number of complex interactions among different enzymes and substrates, combine to make functional analysis challenging. In fact, for some proteins that have been reported to be sumoylated, it is not clear that there is a function, or even that the protein

is really sumoylated under endogenous expression levels of SUMO pathway enzymes.

The most important experiment in studying the function of SUMO conjugation to a particular protein is mutational elimination of the SUMO attachment site(s). This is usually done by mutating the attachment-site lysine, but because lysines can also serve as attachment sites for other modifications, the assignment of any effects to SUMO is more convincing if mutations at other positions in the ΨKXE motif show similar effects. Overexpression, dominant negative, or knockdown experiments involving SUMO pathway enzymes can complement these results, but it is imperative that such experiments be done with both wild-type substrate and the substrate that cannot be sumoylated, to confirm that any effects are direct. Often the same effect is seen whether or not the SUMO attachment site in the protein being studied is present, suggesting that the effect involves sumoylation of another protein in the same pathway.

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## Transcription

Many of the known substrates of SUMO in mammalian systems are involved in gene expression and include transcriptional activators, repressors, coactivators, corepressors, and components of large subnuclear structures called PML nuclear bodies (PML NBs), PODs, or ND10. Two recent reviews address the role of SUMO in transcription (4, 5). For simplicity, the transcriptional effects of SUMO can be divided into two groups: those that are likely to involve sumoylated transcription factors bound to a particular promoter and those that involve PML NBs (4). The activities of many transcription factors are regulated by association with PML NBs, and assembly of PML NBs requires sumoylation of the PML protein. Thus, changes in the level of PML sumoylation have broad effects on transcription by several pathways.

Although direct evidence of sumoylated transcription factors bound to promoters has not yet been obtained, it is becoming clear that the presence of SUMO at a promoter represses trancription. Mutations that prevent SUMO attachment to the transcription factors Elk-1, Sp-3, SREBPs, STAT-1, SRF, c-myb, C/EBPs, to the androgen receptor, or to the coactivator p300 all increase transcription from responsive promoters, consistent with a negative role for SUMO in gene expression (86, 92, 127, 131, 133–138). Some other interesting examples of this effect are several transcription factors with "synergy control motifs," which were originally identified in the glucocorticoid receptor (GR) as peptide motifs that reduce GR-dependent transcription from promoters containing multiple GR binding elements (139). Mutating these motifs does not affect transcription from promoters with a single element. The critical feature of synergy control motifs is a ΨKXE sequence, and these sites are sumoylated, suggesting that SUMO attachment reduces the positive synergistic effect of having multiple receptors bound to the same promoter (139–141). Sumoylation of the progesterone receptor (PR) is also involved in complex negative regulatory interactions in

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which one isoform of PR, PR-A, can "transrepress" the transcriptional activity of the other isoform PR-B or of the estrogen receptor (142).

A possible clue to the mechanism of SUMO in transcriptional repression is that targeting SUMO itself to a promoter, by fusing it to a DNA binding domain, is sufficient to reduce promoter activity (127, 131). Because it is unlikely that SUMO per se has this activity, this result suggests that SUMO recruits other factors that repress transcription. Candidates for such factors include HDACs, the repressor protein Daxx, the NuRD complex component CHD3/ZFH, and PIAS proteins. HDAC6 binds to the repressor domain of p300 only when it is sumoylated. Furthermore, si-RNA-mediated knockdown of HDAC6 relieves SUMO-dependent transcriptional repression by p300, consistent with a model where SUMO attenuates transcription through recruitment of HDAC6 (138). Daxx and CHD3/ZFH both bind directly to SUMO, and both also associate with HDACs and are involved in transcriptional repression (72, 143–145). Intriguingly, some PIAS proteins interact with HDACs, and PIAS proteins also bind directly to SUMO and sumoylated proteins (72, 92, 146, 147). In fact, PIASy actually binds more tightly to SUMO-Sp3 than it does to unmodified Sp3 (92), which it targets for sumoylation, suggesting that PIASy may also function downstream of Sp3 sumoylation. When different PIAS proteins are tethered to promoters by fusion to DNA binding domains, some have negative effects on transcription, while others have positive effects (148). A distinct mechanism for SUMO in transcriptional repression involves sumoylation of HDACs themselves. HDAC1 and HDAC4 are both sumoylated, and sumoylation enhances their transcriptional repression activities (67, 149, 150).

Although SUMO attachment to most transcription factors results in repression, SUMO apparently has positive effects on transcriptional activation by the heat shock factors HSF1 and HSF2 and the $\beta$-catenin activated factor Tcf-4. HSF1 is sumoylated in response to heat shock, coinciding with HSF1 activation (129, 151), and, remarkably, sumoylation promotes binding of both HSF1 and HSF2 to DNA in vitro (151, 152). However, it is not yet clear whether this mechanism operates in vivo (129). Tcf-4-dependent transcription is activated by coexpression of $\beta$-catenin and PIASy, and this activation is reduced when Tcf-4 lacks SUMO attachment sites, suggesting that sumoylation activates Tcf-4 (153).

## PML Nuclear Bodies

Other effects on transcription are mediated by PML NBs, whose central component is the PML protein. PML was discovered because the t(15;17) chromosomal translocation that causes acute promyelocytic leukemia (APL) generates a fusion between PML and the retinoic acid receptor (RAR$\alpha$) [reviewed in (154, 155)]. Normal interphase cells have 5–10 PML NBs per nucleus, but NBs are disrupted by many viruses and by expression of the PML-RAR$\alpha$ fusion. PML -/- mice are viable but vulnerable to infection and to developing tumors, while PML-/- cells in culture are radiation resistant and defective in p53-induced apoptosis. A number of other proteins also localize to PML NBs; these include

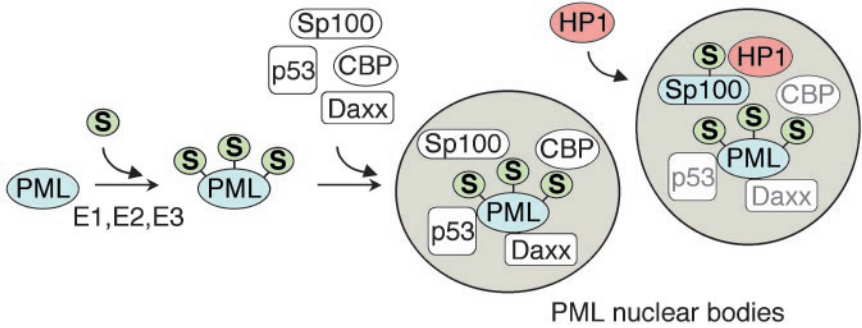


**Figure 3** Assembly of PML nuclear bodies (NBs). Attachment of SUMO (S) to PML promotes formation of PML NBs and recruitment of associated proteins. Sumoylation of associated proteins may allow additional proteins (e.g., HP1) to bind.

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the tumor suppressor p53, the Bloom Syndrome gene product BLM, the coactivator CBP, and Daxx, a transcriptional repressor that has been implicated in apoptosis. Two hypotheses regarding the function of NBs are that they are storage depots for nuclear factors or that they are the site of specific activities, such as modification or assembly of transcription factors. For example, there is evidence that acetylation of p53 by CBP takes place in PML NBs (156).

PML is covalently modified by SUMO at three sites (117, 157, 158), and sumoylation of PML is essential for formation of morphologically normal NBs and for recruitment of interacting proteins. When PML lacking SUMO attachment sites is introduced into PML -/- cells, the mutant PML protein forms aggregates, and many of the interacting proteins, including Sp100, CBP, ISG20, Daxx, and SUMO-1, fail to colocalize with either the PML or with each other (117, 157–161) (Figure 3). In several situations, higher levels of PML-SUMO conjugates correlate with enhanced PML NB formation. Arsenic trioxide, which can be used to treat APL, promotes both sumoylation of PML and reorganization of NBs (157). The converse effect is seen early in infection by many viruses, where disruption of PML NBs takes place simultaneously with desumoylation of PML [reviewed in (162, 163)].

Many of the other proteins that localize to PML NBs also become sumoylated. Curiously, most of these proteins still localize to PML NBs even if their sumoylation sites are mutated, suggesting that sumoylation of these proteins has some purpose other than to promote association with NBs. Proteins for which this is true include p53, LEF1, Sp100, Daxx, SRF1, and the cytomegalovirus proteins IE1 and IE2 (35, 64, 115, 121, 137, 164–167). One possible explanation for these results is that sumoylation of different proteins may produce a hierarchy of interactions: Sumoylation of PML could allow binding of one set of proteins, and sumoylation of these proteins could promote binding of another layer of proteins. For example, sumoylation of Sp100 enhances binding to the heterochromatin protein HP1 in vitro, suggesting that Sp100 sumoylation may recruit HP1 to NBs

(168) (Figure 3). A second possibility is that sumoylation of different proteins creates a web of cooperative interactions, and loss of some of them is not sufficient to destabilize the whole structure (4). It is also conceivable that proteins in NBs may be sumoylated somewhat nonspecifically because high levels of sumoylation occur in NBs or because NB proteins are protected against desumoylating activities. However, arguing against this, mutant versions of Sp100 and CMV IE1 that do not localize to NBs are still sumoylated (115, 169), suggesting that they are specifically targeted for sumoylation.

Changes in the levels of various components of the SUMO pathway can have dramatic effects on the structure of PML NBs, with correspondingly dramatic effects on transcription, probably through sequestration and release of various NB-associated factors. For example, sequestration of the repressor protein Daxx by conditions that promote PML NB formation leads to activation of promoters that are otherwise repressed by Daxx (170–172). Another example involves c-jun-dependent transcription, which is strongly induced by overexpression of the SuPr-1 isoform of the SUMO isopeptidase SENP-2 (110). This induction does not depend on sumoylation of c-jun, but of PML, and does not take place in cells expressing only unsumoylatable PML. Paradoxically, SuPr-1 reduces PML sumoylation and disrupts PML NBs. This result suggests that c-jun-dependent transcription may be induced by a factor that is activated and sequestered in SUMO-PML-containing NBs but that is then released in greater quantities when PML NBs are disrupted by SuPr-1. In addition, overexpression of PIAS proteins has many transcriptional effects, and although it has not been tested in most cases, it seems likely that some of these effects are mediated by changes in PML NBs.

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## Chromosome Organization and Function

Genetic studies of SUMO pathway function in model organisms indicate a role for SUMO conjugation in higher-order chromatin structure and in chromosome segregation, but the molecular basis of these effects is largely unknown. *S. pombe* strains lacking SUMO conjugation, although viable, grow very poorly, are sensitive to DNA damaging agents, have a high frequency of chromosome loss and aberrant mitosis, and develop elongated telomeres (32, 54, 56). Furthermore, a mutant in the *D. melanogaster dpias* gene was isolated as a suppressor of position effect variegation, an effect in which heterochromatin induces transcriptional silencing of adjacent loci (74). *dpias* mutants also have chromosome condensation defects, aberrant chromosome segregation, high frequency of chromosome loss, and defects in telomere clustering and telomere-nuclear lamina associations (74). The *S. cerevisiae ulp2*Δ strain also has a number of phenotypes indicating genomic instability and is defective in targeting the condensin complex, which is required for chromosome condensation, to rDNA repeats (96, 102, 103).

Several lines of evidence implicate SUMO in kinetochore function. SUMO was first identified in yeast as a high-copy suppressor of mutations in the *MIF2*

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gene, which encodes a centromere-binding protein related to vertebrate CENP-C (173). CENP-C mutants are also suppressed by overexpression of SUMO (174). In addition, SUMO localizes at or adjacent to the kinetochore in mammalian cells, and a number of proteins associate with both centromeres and PML NBs, raising the possibility of a common, SUMO-related mechanism (175–178). The best characterized centromere function involves *S. cerevisiae* strains lacking the SUMO isopeptidase Ulp2, which exhibit premature separation of a section of the chromosome near the centromere prior to mitosis (179). *ulp2Δ* strains contain elevated levels of sumoylated topoisomerase II (Top2), and mutating the SUMO attachment sites in Top2 suppresses not only this precocious chromosome separation phenotype but also the temperature sensitivity of *ulp2Δ* mutants, suggesting that these phenotypes result in part from excess SUMO conjugation to Top2.

## DNA Repair

Specific roles for SUMO in two DNA repair pathways have been described, and there are indications that SUMO also acts in other repair pathways. An elegant study focuses on the sumoylation of thymine DNA glycosylase (TDG), a base excision repair enzyme that removes thymine or uracil from T-G or U-G mismatched base pairs (36). The product of the TDG reaction is an abasic site, which is then repaired by downstream enzymes. In vitro, unmodified TDG carries out only a single round of base removal because the enzyme binds tightly to the abasic site that is produced by the reaction. In vivo this interaction probably facilitates transfer of the abasic site to the downstream machinery for completion of repair. SUMO-TDG, in contrast, catalyzes multiple rounds of base removal in vitro, indicating that it is not as strongly inhibited by its product as is unmodified TDG. Furthermore, SUMO conjugation to TDG is stimulated by DNA and by APE1, a downstream enzyme that processes abasic sites. These data suggest a model in which unmodified TDG cleaves the mismatched T or U and then, coordinated with recruitment of the downstream enzymes to the site, is sumoylated, released, and then desumoylated, regenerating the high-affinity form to carry out the next cycle of catalysis (36) (Figure 4*a*).

SUMO may also participate in maintaining the activity of DNA topoisomerase I (TOP1) and topoisomerase II, which are both sumoylated in mammalian cells in response to topoisomerase inhibitors (180, 181). A TOP1 mutant lacking the active site is also constitutively sumoylated in the absence of inhibitors (182), suggesting that some feature of the inactive protein promotes its sumoylation. Upon treatment of cells with the TOP1 inhibitor camptothecin, wild-type TOP1 clears from the nucleoli and disperses throughout the nucleus, whereas TOP1 lacking the SUMO attachment sites remains in the nucleoli (183), indicating that sumoylation may regulate TOP1 localization or may increase its activity.

SUMO also participates in the yeast postreplication repair system, which repairs DNA lesions during the G2 phase of the cell cycle (24). A critical element of this system is the attachment of Ub, either as mono-Ub or as a Ub chain, to

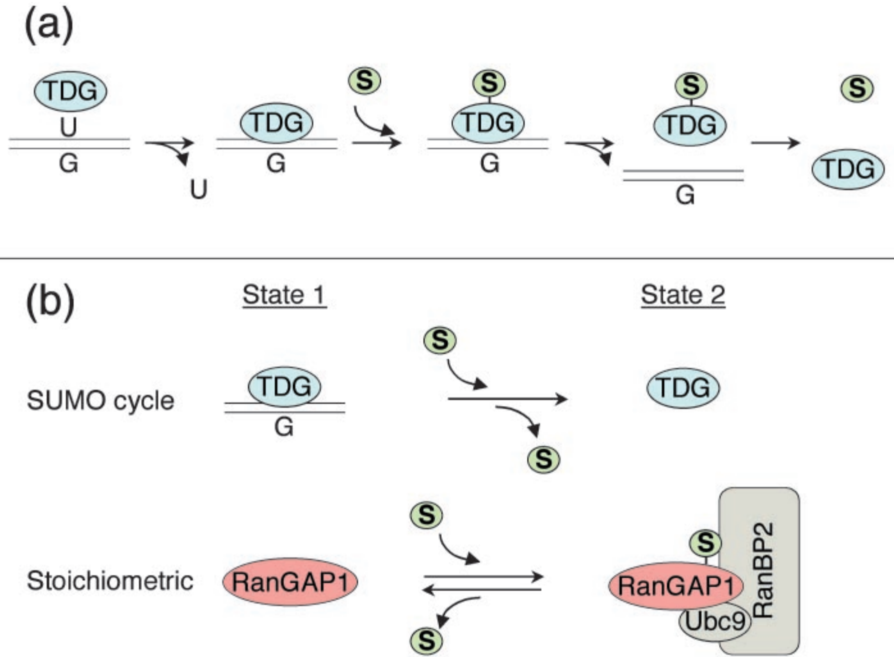


**Figure 4** Stoichiometric versus cycling mechanisms for SUMO. (*a*) A model for the role of SUMO (S) in the thymine DNA glycosylase (TDG) reaction (36). (*b*) SUMO may be able to work through a sumoylation-desumoylation cycle in which SUMO promotes a change in the substrate that persists after desumoylation, or stoichiometrically, such that desumoylation restores the original state. Examples are described in the text.

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the proliferating cell nuclear antigen (PCNA) at $Lys^{164}$. SUMO competes for attachment to this lysine and can also be attached at a second site. As would be expected if SUMO is blocking ubiquitylation, genetic evidence indicates that SUMO conjugation inhibits damage-induced DNA repair and mutagenesis (24, 184). PCNA is sumoylated most heavily during the S phase of the cell cycle; this may suggest that sumoylation prevents inappropriate recruitment of postreplication repair enzymes during the wrong phase of the cell cycle. Interestingly, either sumoylation or mono-ubiquitylation of PCNA can participate in spontaneous mutagenesis by this pathway (184), suggesting that SUMO can also affect PCNA function independently of Ub.

## Nuclear Transport

Investigators studying nuclear transport were the first to discover that SUMO modifies other proteins when they isolated sumoylated RanGAP1, which is the most abundant SUMO-1 conjugate in vertebrate cells (22, 23). RanGAP1 is the

GTPase activating protein for the small GTPase Ran, which plays a central role in nucleocytoplasmic transport and also participates in several events during mitosis (185). It is not clear what role sumoylation of RanGAP1 plays in nuclear transport. SUMO-RanGAP1 binds tightly to the nuclear pore complex (NPC) by participating in a stable trimeric complex with Ubc9 and the IR domain of RanBP2/Nup358 (22, 89, 90). This tightly bound RanGAP1 is crucial in nuclear import assays in vitro, and soluble RanGAP1 cannot substitute for it (22). However, plant and yeast RanGAPs are not sumoylated, and yeast RanGAP localizes to the cytoplasm, indicating that in yeast, nuclear transport does not depend on RanGAP localization at the NPC. Another possible function of RanGAP1 sumoylation is that it could participate in the mitotic functions of Ran. During mitosis, SUMO-RanGAP1 localizes to the mitotic spindle and associates most strongly with the kinetochores. RanGAP1 that cannot be sumoylated does not associate with spindles (176). These results may indicate a centromere-associated function for SUMO-RanGAP1.

SUMO conjugation to proteins other than RanGAP1 also affects nuclear versus cytoplasmic localization. Even though yeast RanGAP is not sumoylated, nuclear import of certain yeast proteins is impaired in SUMO pathway mutants (186). This effect could involve sumoylation of other nuclear transport factors or of the cargo proteins. In mammalian cells, the presence of the E3 RanBP2 on the cytoplasmic site of the NPC and the SUMO isopeptidase SENP2 on the nucleoplasmic side suggests a model in which proteins might be rapidly sumoylated and desumoylated as they are imported into the nucleus (22, 23, 99, 100). There is no direct evidence for this idea, but the SUMO attachment sites in several proteins are required for their nuclear localization (93, 122, 124, 187). However, it is not clear whether SUMO affects nuclear transport or nuclear retention. The opposite effect on nuclear localization has also been seen: Sumoylation of TEL and *Dictyostelium* MEK1 is associated with their export to the cytoplasm (188, 189).

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## Sumoylation of Nonnuclear Proteins

Most SUMO conjugates are nuclear proteins, and it is likely that most of the major functions of SUMO take place in the nucleus. However, there are several cytoplasmic SUMO conjugates. The most prominent example is of course SUMO-RanGAP1, which is found on the cytoplasmic fibrils of the nuclear pore complex. Others include the yeast septins, which form a filamentous structure at the yeast bud neck, the glucose transporters GLUT1 and GLUT4 (40, 190, 191), and the signaling proteins I$\kappa$B$\alpha$, Axin, and *Dictyostelium* MEK1.

## Signal Transduction Pathways

Stimulation of the inflammatory response pathway leads to activation of the transcription factor NF$\kappa$B by promoting Ub-dependent degradation of the NF$\kappa$B inhibitor I$\kappa$B. SUMO conjugation to I$\kappa$B$\alpha$ can inhibit this step because SUMO

is linked to the same Lys where Ub would be attached, thereby preventing IκBα degradation (25). Consistent with a role for SUMO in stabilizing IκBα, SUMO overexpression inhibits NFκB-dependent transcription in mammalian cells (25). Curiously, SUMO has the opposite effect on the orthologous pathway in *Drosophila*, where sumoylation of the NFκB ortholog Dorsal apparently promotes its import into the nucleus and transcriptional activity (192).

Another example of a role for SUMO in signal transduction is found in *Dictyostelium*, where a MAP kinase pathway controls chemotaxis and aggregation in response to extracellular cAMP (189). Within 15 s after cAMP addition, the MAPK kinase MEK1 becomes sumoylated, and the initially nuclear MEK1 and SUMO localize to the plasma membrane. It is not clear whether SUMO enhances nuclear export or plasma membrane association of MEK1. Simultaneously, the downstream MAPK ERK1 relocalizes from the cytoplasm to the plasma membrane, suggesting that ERK1 activation takes place at the plasma membrane. Strikingly, by 3 min after pathway activation, MEK1 has been desumoylated, and MEK1 and SUMO both disappear from the plasma membrane.

The SUMO pathway also affects signaling dependent on Axin, a protein that serves as a scaffold for enzymes in the Wnt pathway and participates in activation of the JNK MAP kinase. Axin is sumoylated at two sites at its extreme C terminus (119), and deletion of these sites eliminates MEKK1-dependent JNK activation but has no effect on Wnt signaling. Axin also interacts with two isoforms of the isopeptidase SENP-2, Axam and Axam2, and expressing either of these inhibits Wnt signaling, although the mechanism is not clear (111, 112).

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# MECHANISMS OF SUMO ACTION

## SUMO's Interactions With the Ub-Proteasome Pathway

One way SUMO affects the function of its substrates is by preventing ubiquitylation at specific lysine residues. PCNA, Smad4, and IκBα are all examples of substrates where a single lysine residue can be either sumoylated or ubiquitylated. However, it is still not clear in these cases whether SUMO exclusively regulates ubiquitylation or whether it also has a distinct function. The model that sumoylation acts solely by blocking ubiquitylation is perplexing, because often very little of the protein is sumoylated. For example, only a small fraction of IκBα is sumoylated in unstimulated cells, so that upon activation of the inflammatory response pathway, most of the NFκB in the cell could still be activated via degradation of the remaining unsumoylated IκBα. One possible answer to this dilemma is that SUMO may act primarily by shutting off the inflammatory response, rather than by modulating its activation (124). Hypoxia induces proinflammatory genes through Ub-dependent degradation of CREB (cAMP response element binding protein). CREB can also be sumoylated and is

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stabilized by SUMO overexpression. Strikingly, hypoxia induces ubiquitylation of CREB within one hour, but it induces sumoylation of both CREB and I$\kappa$B$\alpha$ slowly, with maximal sumoylation after 24–48 h (124). This late induction of sumoylation is consistent with a role for SUMO in resolution of the response.

In other cases, SUMO also appears to have a separate function in addition to preventing ubiquitylation. The transcription factor Smad4 is protected from Ub-dependent proteolysis by attachment of SUMO at its ubiquitylation site, but there is also evidence that sumoylation separately promotes nuclear retention of Smad4 (26). In another example, sumoylation of PCNA inhibits Ub-dependent postreplication DNA repair, consistent with a function for SUMO in blocking ubiquitylation. However, sumoylated PCNA can itself promote spontaneous mutagenesis through the postreplication repair pathway (184), indicating an independent role for SUMO.

SUMO also interacts with the Ub-proteasome pathway by other uncharacterized mechanisms. Sumoylation inhibits degradation of c-myb but not by competing for the ubiquitylation site (134). In contrast, SUMO conjugation coincides with degradation of both PML and the PML-RAR$\alpha$ fusion protein. Agents such as arsenic trioxide induce both sumoylation and proteasome-dependent degradation of PML and PML-RAR$\alpha$, and the SUMO attachment sites are required for this degradation (161, 193). Of course, if these sites were also used as ubiquitylation sites, the same result would be obtained. Arsenic trioxide also enhances recruitment of the 11S proteasome regulator to PML NBs (161).

## Sumoylation Modulates Interactions of Substrate

The most common mode of SUMO action is to alter substrate binding interactions with other macromolecules. Three nonmutually exclusive models for this are (*a*) the linked SUMO itself could interact with other proteins; (*b*) both SUMO and the substrate could contribute determinants of the interaction surface; or (*c*) SUMO could alter the conformation of the substrate, exposing or hiding binding sites within the modified protein. Several of the proteins isolated in the yeast two-hybrid screen with SUMO do bind SUMO noncovalently in pull-down assays. A number of these, including HIPK2 (homeodomain-interacting protein kinase 2), the cytomegalovirus protein IE2, and PIAS proteins, contain a SUMO interacting motif (SIM), which is likely to mediate this interaction. The function of SIMs has not been fully investigated, but the SIM in PIASy is involved in its localization and transcriptional effects (64), and the SIM in HIPK-2 is required for HIPK-2-dependent disruption of PML NBs (194), demonstrating that these motifs have relevant physiological functions. Many of the proteins that interact with SUMO noncovalently, such as TDG, Daxx, CMV IE2, and Dnmt3b, are also covalently modified by SUMO (36, 144, 164, 195). This ability of proteins both to be sumoylated and to interact noncovalently with SUMO may enhance complex formation between various sumoylated proteins, as in PML NBs. However, there are very little data on the prevalence and function of direct noncovalent interactions with conjugated SUMO, and it seems likely that other

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interactions involving the substrate would also be required for the effects of SUMO to be substrate-specific.

For most of the substrates that have been characterized, changes in binding capabilities are a collaboration between SUMO and the substrate. Sumoylation alters the DNA-binding characteristics of TDG, reducing its affinity for the abasic sites that are the products of its reaction (36). Two ideas for the way this might take place are that SUMO attachment could induce a conformational change in TDG or that SUMO could act more directly, possibly blocking access to the DNA by steric hindrance. In another example, association of the SUMO-RanGAP1 conjugate with RanBP2 requires both SUMO and sequences in RanGAP1. Unsumoylated RanGAP1 does not bind RanBP2, but there is also one RanGAP1 deletion mutant that is sumoylated properly but still does not associate with RanBP2 (90). This result shows that the presence of SUMO is not sufficient for binding to RanBP2; the binding determinant must include sequences in RanGAP1. Supporting this interpretation, free SUMO does not compete with SUMO-RanGAP1 for binding to RanBP2 (22). These results could be explained either by a model in which SUMO induces a conformational change in RanGAP1 to expose a RanBP2 binding-site that is entirely in RanGAP1 or by a model in which RanBP2 interacts with elements in both RanGAP1 and SUMO (90).

## Stoichiometric Versus Cycling Roles for SUMO Conjugation

A notable feature of the SUMO system is that SUMO is often attached to only a few percent or less of a given protein. The only clear exception is RanGAP1, which is ~50% modified in most cells (22, 23). Therefore an important unresolved question is how SUMO can affect protein function when only a very small fraction is modified. One possibility is that SUMO could act on a subpopulation of a protein that is different structurally or functionally from the rest of the pool of that protein. For example, it is possible that some transcription factors are preferentially sumoylated when they are bound to certain promoters. Another possibility is that SUMO conjugation could be acting through a cycle of sumoylation and desumoylation, rather than by persistent attachment of SUMO to the substrate. In this model, SUMO attachment would promote a single event, whose consequences would persist after desumoylation. The role that has been proposed for SUMO in TDG function is an example of a such a cycle (36) (Figure 4*a*). Unmodified TDG removes the thymine or uracil at a mismatched site and then remains bound until it is sumoylated. The sumoylated TDG releases from the abasic site and is then desumoylated to prepare it for the next round of high-affinity binding. This cycle converts the DNA-bound form of TDG, state 1, to the unbound form, state 2, where neither of the two states is modified by SUMO (Figure 4*b*). In this way, the whole population of a protein could be affected by sumoylation, but very little of it would be modified at a given time. It easy to imagine how a sumoylation-desumoylation cycle could act in other situations as well, possibly by promoting protein interactions, inducing confor-

mational changes, or even stimulating other protein modifications that would then be maintained after removal of SUMO. Many of the functions of Ub, including the proteasome pathway and the sorting of membrane proteins in the endosomal system, are carried out by a cycle of ubiquitylation and deubiquitylation. This SUMO cycle model contrasts with a model in which SUMO acts stoichiometrically (Figure 4*b*). Here, attachment of SUMO alters the state of the substrate, and desumoylation returns it to its original state. This is likely to be the case with RanGAP1; the unmodified form localizes to the cytoplasm, state 1, while the sumoylated form associates tightly with the nuclear pore, state 2.

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## CONCLUDING REMARKS

Work over the last several years has shown SUMO to be a remarkably versatile regulator of protein function, both in the number of different biological pathways that it affects and in the different sorts of mechanisms by which it controls the activities of other proteins. Many fundamental questions remain to be answered about both the biological function of SUMO and its mechanism of action. Why is SUMO essential for viability of most eukaryotic cells? What role does it play in maintaining chromosome structure? What are the substrates whose sumoylation participates in these processes? How are sumoylation and desumoylation regulated? How does SUMO alter binding properties of proteins?

There are also fields in which we are catching only our first glimpses of a role for SUMO, as in the pathogenesis of several neurodegenerative diseases. The difficulties associated with detecting SUMO-modified proteins have delayed recognition of the widespread participation of SUMO in cellular processes, and it is likely that as these difficulties are overcome, even more roles for SUMO will be discovered.

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