Published in final edited form as:

Nat Rev Mol Cell Biol. 2012 December ; 13(12): 755–766. doi:10.1038/nrm3478.

Function and Regulation of SUMO Proteases

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Abstract

Covalent attachment of small ubiquitin-like modifier (SUMO) to proteins is highly dynamic, and both SUMO-protein conjugation and cleavage can be regulated. Protein desumoylation is performed by SUMO proteases, which control cellular mechanisms ranging from transcription and cell division to ribosome biogenesis. Recent advances include the discovery of two novel classes of SUMO proteases, insights regarding SUMO protease specificity, and revelations of previously unappreciated SUMO protease functions in several key cellular pathways. These developments, together with new connections between SUMO proteases and the recently discovered SUMO-targeted ubiquitin ligases (STUbLs), make this an exciting period for the study of these enzymes.

Introduction

Cellular proteins are often covalently modified following their translation, providing organisms with added control of protein activity, localization or stability. Among the many known post-translational protein modifiers are other proteins, most commonly ubiquitin or one of several ubiquitin-like proteins (UBLs). Ubiquitin and UBLs share a common fold, known as the β -grasp fold, and a conserved enzymatic strategy for their activation and attachment to substrates¹. The UBL known as small ubiquitin-related modifier (SUMO) is attached to a multitude of proteins in all species that have been analyzed, including *Saccharomyces cerevisiae*^{2–5}, *Arabidopsis thaliana*^{6, 7} and humans^{8, 9}. Correspondingly, sumoylation is known to affect numerous cellular processes. Although SUMO attachment to proteins can have distinct physiological consequences, its primary molecular function is to control interactions of the modified proteins with other proteins. Many cellular proteins bear SUMO-interacting motifs (SIMs) that bind SUMO non-covalently¹⁰ [Box 1].

SUMO attachment to proteins requires ATP and typically involves three enzymes: a heterodimeric SUMO-activating enzyme (E1) that activates the SUMO C-terminal carboxyl group and then forms a thioester with the SUMO C-terminus, a SUMO-conjugating enzyme (E2), and one of several SUMO ligases (E3s)¹¹ [FIG. 1]. Like ubiquitin, SUMO is usually ligated to substrate lysine side chains, forming amide (isopeptide) linkages. It may modify a single substrate lysine or multiple lysines (multisumoylation) or form SUMO chains on its substrates (polysumoylation). Yeast has only one form of SUMO (encoded by *SMT3*), but most vertebrates express at least three SUMO isoforms, SUMO1, SUMO2, and SUMO3, each encoded by a distinct gene. SUMO2 and SUMO3 are nearly identical and will hereafter be referred to as SUMO2/3 when isoform type needs to be specified. SUMO1 is ~48% identical to SUMO2/3. All SUMO isoforms are synthesized from C-terminally extended precursors that first need to be processed to their active forms, which terminate with a pair of glycine residues [FIG 1b]. A fourth SUMO paralogue (SUMO4) gene is present in

humans, but it might not encode a molecule that is covalently coupled to proteins because its precursor does not seem to be processed *in vivo*¹².

Deconjugation of SUMO from proteins is carried out by enzymes referred to generically as SUMO proteases [FIG. 1]. These enzymes cleave precisely between the terminal glycine of SUMO and the substrate lysine [FIG. 1c]. Certain SUMO proteases are also responsible for SUMO precursor maturation and thus indirectly affect SUMO conjugation. The first SUMO protease to be described was the *S. cerevisiae* protein Ulp1 (UBL-specific protease 1)¹³. By comparing the sequence of the Ulp1 catalytic domain to sequence databases, a second budding yeast SUMO protease (Ulp2)¹⁴ and putative SUMO proteases in many other organisms were identified^{13, 15}. The first human protein to be described as similar to Ulp1 was confirmed as a SUMO-specific protease and named sentrin-specific protease 1 (SEN1; SUMO is also known as sentrin)¹⁶. More extensive database searches identified additional putative human SENPs¹⁷, and subsequent studies have shown that humans express six SUMO proteases of the SENP class: SENP1–SEN3 and SENP5–SEN7. Moreover, recent studies have identified three new SUMO proteases in humans, desumoylating isopeptidase 1 (DeSI1), DeSI2¹⁸ and ubiquitin-specific protease-like 1 (USPL1)¹⁹, which share little sequence similarity with the Ulp/SEN1 protease class.

Recent investigations into the biochemical activities and subcellular localization of SUMO proteases have contributed much towards our understanding of the specificity of these enzymes. New functions of SUMO proteases continue to be unraveled and intriguing examples of SUMO protease regulation have recently been uncovered. In this review we incorporate discussion of the newly discovered classes of SUMO proteases and the novel features found for the established Ulp/SEN1 enzymes into a broader appraisal of the SUMO protease field.

Unexpected Variety in SUMO proteases

The Ulp/SEN1 enzymes were the only known structural class of SUMO proteases until the very recent characterization of DeSI1 and DeSI2¹⁸ and USPL1¹⁹. All three groups are cysteine proteases with a papain-like proteinase fold. The identification of three distinct classes of SUMO proteases is reminiscent of the diversification seen for deubiquitylating enzymes (DUBs), which fall into five known structural classes²⁰. The expansion of paralogues within the DUB superfamily, however, is far greater, with ~100 DUBs predicted for humans, none of which resembles the Ulp/SEN1 proteases. USPL1, however, as its name suggests, does distantly resemble the USP class of DUBs¹⁹. USPL1 is found throughout the vertebrates and in several invertebrates. The characterization of USPL1 in zebrafish (*Danio rerio*) provides evidence that the SUMO protease function of USPL1 is conserved in evolution. DeSI proteins are distinct from the known DUBs and Ulp/SEN1s. Instead, they belong to the PPPDE (permuted papain fold peptidases of ds-RNA viruses and eukaryotes) class of proteases²¹, which do not have a representative in *S. cerevisiae* but are found in other fungi, including *Schizosaccharomyces pombe*. Whether non-mammalian PPPDE proteases function as SUMO-specific proteases has not been tested.

That USPL1 most closely resembles a family of deubiquitylating enzymes but is in fact a SUMO-specific protease underscores the importance of biochemical characterization for putative proteases. A similar surprise was observed with the characterization of DEN1, which was originally annotated SENP8 due to its sequence similarity to the Ulp/SEN1 proteases, but was later found to be specific for the UBL Ned1 rather than SUMO^{22–24}.

Structures of SUMO proteases

Most of the known SUMO proteases have substantial noncatalytic regions [FIG. 2a]. For the well-characterized Ulp/SENp proteases, N-terminal regions, although poorly conserved, regulate their intracellular localization^{25–30} [TABLE 1]. Yeast Ulp2 and multiple metazoan SENPs also contain putative SIMs in their non-catalytic regions [FIG. 2a]. A putative SIM can also be found in DeSI1, although in this case within the catalytic domain. If they are indeed functional, these SIMs might increase enzyme affinity for SUMO or SUMO chains or they might help orient the sumoylated protein on the SUMO protease. The catalytic domains of Ulp/SENp enzymes bind SUMO via surfaces that are distinct from SIMs³¹ [FIG. 2b, c].

Insight into the specificity and function of SUMO proteases has been gained through high-resolution structural investigations of the proteases, in some cases in complex with SUMO or SUMO-conjugated substrates. The first SUMO protease structure solved, that of the catalytic domain of yeast Ulp1 in a covalent transition state-like complex with SUMO³¹, revealed that the overall architecture of the Ulp1 catalytic domain resembles that of other cysteine proteases, as had been predicted from the sequence similarity of this domain to the adenovirus processing protease AVP¹³. Specificity for SUMO is achieved through an extensive interface involving unique hydrophilic interactions and numerous salt bridges. The active site pocket orients the catalytic triad residues (order: His-Asp-Cys) for substrate cleavage and positions the SUMO C-terminal diglycine motif in a shallow tunnel formed by two Trp residues — side chains of residues other than glycine would sterically clash with the narrow Trp tunnel. Structures for the catalytic domains of the mammalian SUMO proteases SENP1 and SENP2 are similar to that of Ulp1 and share the Trp tunnel and SUMO-binding surfaces noted for Ulp1 [FIG. 2b–d]^{32–35}. As reviewed by Huang and Schulman³⁶, SENP1 and SENP2 (and likely other members of the Ulp/SENp family) orient the scissile bond in a *cis* configuration, which leads to a kink in the SUMO C-terminal tails for SUMO precursors or in the isopeptide linkage to sumoylated proteins^{33, 34}. These nonprolyl *cis* peptide bonds are rare in proteins and are thought to destabilize the scissile bond and promote cleavage.

The catalytic domains of SENP6, SENP7 and Ulp2 have low sequence identity to the catalytic domain of Ulp1 (less than 30%) but are more similar to one another and thus represent a distinct subgroup of Ulp and SENp enzymes¹³. SENP6 and SENP7 have four conserved loop insertions within the catalytic domain that are absent in the other mammalian SENPs. As revealed in the crystal structure of SENP7, the Loop-1 insertion — which is not conserved in yeast Ulp2 — projects towards the presumed SUMO-binding interface, suggesting a role in binding the SUMO-conjugated substrate [FIG. 2e]³⁷. Consistent with this, *in vitro* activity assays with the mutant SENP7 catalytic domain lacking Loop-1 show that it is less efficient at deconjugating SUMO substrates than the wild-type version. Notably, SENP6 and SENP7 normally have higher activity toward SUMO2/3 than SUMO1 [Table 1]. Paralogue specificity of these proteases could be altered by mutating two key residues on SUMO2 that likely contact Loop-1 of SENP6 and SENP7 to those that are found in SUMO1³⁸. This SUMO2 mutant is no longer efficiently deconjugated by SENP6 or SENP7. Conversely, a mutant SUMO1 that has these two residues replaced by those of SUMO2 was a much better substrate for these SENPs. Thus, contacts between Loop-1 of SENP6 or SENP7 and SUMO2/3 contribute substantially to SUMO paralogue specificity.

Crystal structures of mouse DeSI1 [FIG. 2f] and a truncated human DeSI1 show several interesting features^{39, 40}. The protease forms a dimer in which the active-site residues face a surface groove formed between the two monomers. Curiously, the C-terminal tails of the full-length DeSI1 fold into the groove and seem to block substrate access to the active sites. However, activity assays suggest truncation of the C-terminal tail has no effect on substrate

cleavage rates³⁹. SUMO-modified substrates might be able to displace the C-tails, or the occluded conformation may be a result of crystallization. Whether both active sites must be functional in the homodimer also remains to be determined.

In contrast to the catalytic triad in Ulp1 and SENPs, DeSI1 and DeSI2 have been suggested to have only a catalytic dyad (Cys-His)³⁹. Cys108 and His38 are positioned as in other papain-fold proteases, and indeed, mutagenesis studies show that SUMO substrate cleavage is abolished when these residues are mutated^{18, 39}. A comparative analysis of structures of the Nlp60/P60 cysteine protease superfamily, to which the DeSI enzymes belong, led to the suggestion that another residue in the DeSI enzymes binds a water molecule that in turn helps orient the catalytic His residue⁴⁰. Structural analysis of DeSI1 in complex with a sumoylated substrate should help clarify these mechanistic issues. No high-resolution structure is currently available for USPL1, but its sequence similarity to DUBs of the USP class suggests its catalytic domain will have a comparable architecture¹⁹.

Substrate specificity of SUMO proteases

The specificity of the SUMO proteases has been extensively investigated both *in vitro* and *in vivo* and has been found to be strongly influenced by the localization of the protein [TABLE 1].

Yeast SUMO proteases

The yeast Ulp1 enzyme, unlike Ulp2, shows broad specificity *in vitro*, where it is capable of efficiently cleaving SUMO translational fusions and isopeptide-linked SUMO, including polySUMO modifications^{13, 31, 41}. Ulp1 is largely responsible for SUMO precursor processing *in vivo*, and it also removes post-translational SUMO modifications from protein substrates^{13, 25}. Ulp2 has particularly high activity towards the SUMO-SUMO linkages in polySUMO chains both *in vitro* and *in vivo*, although it also can cleave isopeptide linkages between SUMO and substrates^{14, 41}. Genetic studies using yeast strains with mutations in Ulp1 or Ulp2 indicate that the two yeast SUMO proteases have distinct substrate specificities as unique sumoylated substrates accumulate in each protease-deficient strain, and the mutants have distinct phenotypic defects¹⁴.

Substrate specificity of Ulp1 and Ulp2 is strongly influenced by their restricted cellular localization²⁵. Ulp2 is found throughout the nucleoplasm¹⁴, whereas Ulp1 localizes primarily to the inner surface of the nuclear pore complex (NPC). However, a fraction of Ulp1 is exported to the cytoplasm in a cell cycle-specific manner and desumoylates the heavily sumoylated septin proteins that form filaments at the bud neck of dividing cells^{42, 43}. Localization is dictated by their noncatalytic N-terminal domains: indeed, an N-terminally deleted Ulp1 can localize throughout the cell. When expressed in an *ulp2* null strain, this truncated Ulp1 enzyme partially suppresses the *ulp2* mutant growth defects, such as irreversible arrest in response to DNA damage, and reduces the levels of sumoylated Ulp2 substrates, highlighting the importance of restricting Ulp1 localization in controlling its *in vivo* activity²⁵. Moreover, high levels of Ulp1 activity mislocalized to the nucleoplasm are lethal^{25, 44}. Nuclear localization of Ulp2, in contrast, is crucial for its function²⁶.

Mammalian SUMO proteases

As observed with the yeast SUMO proteases, the *in vivo* specificities of the mammalian SENPs are governed in part by their localization. Most of the SENPs concentrate in the nucleus, with many of them localized to recognizable subnuclear compartments [Table 1]⁴⁵. SENP3 and SENP5 localize to the nucleolus^{27, 46}. SENP6 localizes throughout the nucleoplasm³⁰, but a catalytically inactive SENP6 concentrates in nuclear foci⁴⁷, a fraction of which seem to be PML (promyelocytic leukemia protein) nuclear bodies, consistent with

PML being a substrate of SENP6. Interestingly, SENP2 encodes at least three alternatively spliced mRNA variants and the resulting proteins localize differently, including one variant that localizes to the cytoplasm^{45, 48}. Since SENPs have been visualized in the cytoplasm to some extent^{48–50}, and all are synthesized in the cytoplasm, SENP-mediated desumoylation is likely to occur at some frequency in the cytoplasm.

Deconjugation of sumoylated substrates by individual SENPs *in vitro* is generally not strongly SUMO paralogue-specific, although some of the SENPs exhibit greater activity towards one or another isoform [Table 1]. However, greater paralogue specificity *in vivo* is sometimes observed. Although the basis for the *in vivo* paralogue specificities of SENPs is not completely understood, competition for substrate binding by other cellular proteins is likely to be the explanation in most cases. An interesting example is the nearly exclusive modification *in vivo* of RanGAP1 by SUMO1 rather than SUMO2/3 even though *in vitro* the SUMO isoforms are conjugated to RanGAP1 with similar efficiencies⁵¹. Sumoylated RanGAP1 binds the NPC component Nup358, with SUMO1-RanGAP1 interacting more tightly with Nup358 than SUMO2/3-RanGAP1. This selectively protects the SUMO1-RanGAP1 conjugate from SUMO proteases.

By contrast to deconjugation of sumoylated proteins, SUMO precursor processing by SENPs is often paralogue-specific^{35, 46, 52}. The basis for this discrimination lies in the predicted contacts made between the protease and propeptide residues following the C-terminal diglycine motif³⁵. The propeptide sequences in the SUMO paralog precursors are quite divergent [FIG. 1b], and certain propeptide extensions fit better than others in binding pockets that lie adjacent to the catalytic residues of the protease³⁵. These structure-based studies of precursor processing are currently limited to SENP1 and SENP2. Of the other SENPs, SENP5 has significant SUMO precursor processing activity for SUMO3 and limited activity toward SUMO1⁴⁶, SENP6 and SENP7 do not possess SUMO precursor processing activity³⁷, and SENP3 has not been tested due to the inability to obtain purified, active SENP3 [TABLE 1].

DeSI1 was identified in a screen for proteins that bind to the transcriptional repressor BZEL (BTB-ZF protein expressed in effector lymphocytes)¹⁸. DeSI1 is diffusely localized throughout the cytoplasm and nucleus in 293T cells and desumoylation assays carried out both in 293T cells and with recombinant proteins revealed that DeSI1 specifically desumoylates BZEL. DeSI1 deconjugates both SUMO1 and SUMO2/3 from BZEL and weakly cleaves poly-SUMO2/3 chains^{18, 39}. Importantly, the substrate specificity of DeSI1 is distinct from that of SENPs, as DeSI1 does not desumoylate known substrates of SENPs¹⁸. BZEL is currently the only known substrate of DeSI1 and knockdown of DeSI1 expression in 293T cells does not detectably alter bulk SUMO-conjugate profiles, suggesting a limited number of substrates. One caveat to this conclusion is that the very similar DeSI2, which also concentrates in the cytoplasm, might partially compensate for DeSI1. Therefore, it will be useful to evaluate cellular phenotypes in cells co-depleted for the two DeSI enzymes.

USPL1 was identified as a protein in HeLa cell extracts that crosslinked to either SUMO1 or SUMO3 that had been derivatized at its C-terminus by chemical ligation to vinyl methylester, a thiol reactive functional group that covalently modifies active site cysteines of enzymes¹⁹. USPL1 localizes to Cajal bodies within the nucleus of the cell, which is unique among the known SUMO proteases. Like Ulp1 and certain SENPs, USPL1 shows broad SUMO protease activity *in vitro*. Furthermore, bulk SUMO-conjugates are reduced upon USPL1 overexpression in HEK293T cells. In contrast, knockdown of USPL1 expression does not cause a detectable change in bulk SUMO-conjugate profiles, but it does impair cell growth. Interestingly, supplying an exogenous, non-catalytic form of USPL1 is able to

suppress the growth defects caused by knockdown of endogenous USPL1. Thus, although USPL1 is clearly a SUMO protease, it appears to have an essential role in cell division that is independent of its SUMO protease activity.

Regulation of SUMO proteases

In addition to the constraints placed on SUMO protease activity by their localization or by substrate shielding, other mechanisms of regulating SUMO protease function, particularly in response to environmental stimuli, have been described.

Regulation of transcription

Altered transcription of specific mammalian *SENP* genes is a common mechanism affecting the sumoylation of specific proteins [FIG. 3a]. *SENP1* expression is induced by androgen receptor signaling, and androgen response elements are present in the promoter region of *SENP1*⁵³. The *SENP1* promoter also contains a hypoxia response element, and *SENP1* levels increase upon oxygen deprivation (hypoxia) in a manner dependent on HIF1 α ⁵⁴. SUMO proteases can in turn regulate transcription of other genes (see below), and in some cases, they control their own transcription. During both hypoxia and androgen signaling, *SENP1* is proposed to participate in positive feedback loops wherein *SENP1* has a stimulatory effect on its own transcription in addition to stimulating the transcription of the other targets of these pathways^{53–55}. For example, *SENP1* is proposed to desumoylate HIF1 α (which is sumoylated, ubiquitylated and degraded in response to hypoxia) and enhance its stability, which is consistent with increased sumoylated HIF1 α and decreased expression of HIF1 α target genes in cells lacking *SENP1*⁵⁵. The exact mechanism of feedback control for *SENP1* and androgen signaling remains to be determined⁵³.

SENP2 is also known to regulate its own transcription, but in this case it operates in a negative feedback loop⁵⁶. Following an early increase in *SENP2* mRNA levels in response to genotoxic insults, *SENP2* desumoylates the NF- κ B activator NEMO (NF- κ B essential modulator), which in turn attenuates *SENP2* transcription as the response progresses. As NF- κ B signaling promotes cell survival, this negative feedback loop is one mechanism used by cells to prevent the survival of cells that are damaged beyond repair.

Regulation by post-translational modifications

SUMO proteases are subject to post-translational modifications that regulate their activity and/or stability. *SENP3* is modified by ubiquitin and subsequently degraded by the proteasome [FIG. 3b]. *SENP3* turnover by the ubiquitin-proteasome system (UPS) is accelerated by its interaction with the tumor suppressor p19(Arf)⁵⁷ and dampened by oxidative stress⁵⁸. Under normal growth conditions, *SENP3* is continuously ubiquitylated by the ubiquitin ligase CHIP⁵⁹. Mild oxidative stress leads to association of the chaperone protein Hsp90 with *SENP3*, protecting it from CHIP-mediated ubiquitylation. Interestingly, stabilization of *SENP3* leads to *SENP3* accumulation in the nucleoplasm (outside of the nucleolus, where it is normally localized), and hence *SENP3* can gain access to additional substrates, such as the general transcriptional activator p300, under these conditions⁵⁸. p300 is a co-activator of HIF1 α , and desumoylation of p300 enhances the expression of HIF1 α target genes, which is a known cellular response to mild oxidative stress.

In yeast, Ulp2 accumulates in a (hyper)phosphorylated form specifically during mitosis [FIG. 3c], and this depends on the kinases Cdk1 and Cdc5 (Polo)⁶⁰. Ulp2 is phosphorylated on its noncatalytic C-terminal domain, although the modified residues have not been definitively mapped. Interestingly, two known targets of Ulp2-dependent desumoylation that participate in chromatid cohesion and segregation, Top2 and Pds5, fail to accumulate in

sumoylated form in a *cdc5* mutant, consistent with Polo kinase acting as a negative regulator of Ulp2⁶⁰.

Regulation of SUMO protease activity

The specific activities of selected SUMO proteases are directly affected by certain cellular stresses. One example is during heat shock, which leads to a rapid increase in the sumoylation of many proteins in organisms from yeast to humans⁶¹. A very recent study found that several SENPs are irreversibly inactivated by heat shock, both in cultured cells and *in vitro*⁶². Experiments using purified SENP catalytic domains suggest that this thermal inactivation is due to protein denaturation. Interestingly, SENP6 was more thermotolerant than the other SENPs, perhaps indicating a unique role for SENP6 during heat shock. It is unlikely that the thermal inactivation of SENPs is the only mechanism for cellular sumoylation alterations during heat shock. Another recent report suggests that the increase in sumoylation during heat shock involves changes in the ratio of sumoylated versus unmodified SAE2, one subunit of the dimeric SUMO E1 enzyme⁶³. Heat shock leads to an increase in unmodified SAE2 and a corresponding decrease in sumoylated SAE2, which is less active than unmodified SAE2 in the transfer of thioester-linked SUMO to E2. As suggested by Truong et al., a SUMO protease is likely to remove the inhibitory SUMOs from SAE2 during heat shock⁶³, but this hypothesis remains to be tested. Since only a fraction of SAE2 exists in the sumoylated (less active) form under standard growth conditions, this mechanism may only account for a portion of the cellular sumoylation response to heat shock.

Another potential example of regulatory SUMO protease inactivation is the reversible formation of an intersubunit disulfide between two molecules of SENP1 (and potentially Ulp1 in yeast) in response to oxidative stress⁶⁴. The active site cysteine participates in disulfide formation, and *in vitro* evidence suggests that reversible disulfide formation may protect the enzyme from irreversible oxidative inactivation of the catalytic cysteine.

Cellular roles of SUMO proteases

SUMO proteases affect numerous cellular processes and when mutated, cause a corresponding diversity of defects, including lethality^{13, 55, 65}. Because of their diverse roles, we cannot review all known or suspected SUMO protease functions here, but instead focus on a few illustrative examples. In many cases, the relevant sumoylated substrate or substrates are not known with certainty. This uncertainty relates to two general observations regarding *in vivo* sumoylation. First, at steady state only a small percentage of any given protein substrate is present in its sumoylated form. Second, it is often the case that multiple proteins involved in a particular physiological process or pathway are sumoylated and/or interact noncovalently with SUMO. Therefore, blocking or enhancing the sumoylation of any one protein often does not yield a strong phenotypic deficiency.

Transcription

Numerous transcriptional programmes are regulated by the SUMO pathway, and multiple recent investigations implicate SUMO proteases in the regulation of specific transcription factor activities^{18, 58, 66–75}. Most commonly, Ulp- or SENP-mediated removal of SUMO results in increased transcriptional activity of the target factor. This is presumably due to the variety of factors involved in transcriptional repression, such as histone deacetylases (HDACs), polycomb-related repressors and other co-repressors, that are more effectively recruited by sumoylated transcription factors than their unmodified forms⁷⁶. In the case of the transcription factor Elk1, its sumoylation recruits HDAC2⁷⁷. Sumoylated Elk1 is

desumoylated mainly by SENP1 and SENP1 depletion dampens transcriptional activation mediated through Elk1⁶⁸.

However, exceptions to this generalization are known, as in the case of SENP1-mediated regulation of the Wnt signaling pathway [FIG. 4]⁷². A major regulator of developmental programmes, the Wnt pathway is tightly coordinated to prevent aberrant cell growth and proliferation. The transcriptional regulators TBL1 and TBLR1 were previously reported as activators of the Wnt pathway through their interaction with β -catenin, a transcriptional coactivator⁷⁸. TBL1 and TBLR1 are sumoylated following activation of the Wnt pathway, and sumoylation-deficient mutants of these proteins are unable to activate a Wnt signaling response⁷². Sumoylation of TBL1-TBLR1 was found to promote TBL1-TBLR1 dissociation from the NCoR-HDAC3 corepressor complex and to promote formation of a TBL1^{SUMO}-TBLR1^{SUMO}- β -catenin complex, which is recruited to Wnt target genes by TCF4, a sequence-specific DNA-binding protein. Of the SENP proteases tested, only SENP1 interacts with TBL1-TBLR1, and ectopic expression of SENP1 in 293T cells resulted in desumoylation of TBL1-TBLR1 and decreased activation of Wnt pathway target genes. This effect was abolished with a SENP1 catalytic mutant (SENP1^{C603S}), indicating that the SUMO-deconjugating activity of SENP1 is required. Lastly, shRNA knockdown of SENP1 in 293T cells led to the accumulation of sumoylated TBL1-TBLR1 and enhanced activation of Wnt target genes. These data identify SENP1 as a negative regulator of the Wnt signaling pathway that seems to work by triggering disassembly of a transcriptional co-activator complex.

Cell cycle progression

Ulp1 is essential for progression past the G2/M phase of the cell cycle in budding yeast¹³. Ulp1 is required for SUMO precursor processing, but providing mature SUMO does not reverse the growth defects of a temperature-sensitive *ulp1* mutant. This suggests that these cell cycle defects are caused by the persistent or excess sumoylation of Ulp1 target proteins. The identities of the key sumoylated proteins linked to the cell cycle function(s) of Ulp1 remain unknown, although proteins with roles in cell division, such as the spindle-orientation protein Kar9, have been identified as Ulp1 substrates^{42, 79, 80}.

Yeast Ulp2 also has roles in cell division^{14, 81}. At elevated temperatures and following DNA damage¹⁴, Ulp2 is essential for cell division, functioning in the resumption of the cell cycle after the checkpoint has been turned off or the DNA damage has been repaired^{82, 83}. Under normal conditions, yeast can divide in the absence of Ulp2, but growth is poor, and *ulp2*-deficient cells have abnormal cell size and morphology¹⁴. Poor growth in these cells has been linked to improper centromere cohesion dynamics⁸⁴, in part due to persistent or excess sumoylation of DNA topoisomerase II (Top2). Indeed, *ulp2*-deficient cells that express Top2 that cannot be sumoylated display reduced centromere cohesion defects and show improved growth at elevated temperature⁸⁴. Poor growth in *ulp2*-deficient cells is also thought to be due in part to enhanced spontaneous homologous recombination (HR); these HR defects could be due to altered sumoylation of several substrates, as many proteins within nuclear recombination foci are sumoylated⁸⁵.

Poor growth in yeast cells lacking Ulp2 has also been linked to abnormal mitotic spindles¹⁴, but the basis of this is not clear. However, studies of human SENP6, which resembles Ulp2 in structure and function, have shed light on a role for SUMO2/3 deconjugation during kinetochore assembly⁸⁶. Kinetochores are large protein assemblies that physically link spindle microtubules to the centromeres of chromosomes during mitosis. A kinetochore subcomplex known as the CENP-H/I/K complex is less abundant in cells depleted of SENP6, and these cells mis-segregate chromosomes at an elevated rate compared with control cells. Interestingly, co-depletion of a ubiquitin ligase (RNF4; see below) that

ubiquitylates polysumoylated proteins stabilizes the CENPH/I/K complex and partially restores accurate chromosome alignment during metaphase. Since polysumoylated CENPH and CENPI accumulate in these SENP6- and RNF4-depleted cells, the authors propose that under normal conditions RNF4 targets CENPH- and CENPI-containing complexes that have deviated from their correct assembly pathway. Thus, SENP6 is thought to ensure proper kinetochore assembly on chromosomes by only desumoylating correctly assembled kinetochores leaving off-pathway intermediates to be ubiquitylated by RNF4 and ultimately degraded.

Human SENP5 also has a role in cell division, as knockdown of SENP5 results in decreased cell proliferation and abnormal nuclear morphology⁴⁶. Understanding the mechanistic basis of these defects awaits further investigation.

Ribosome biogenesis

Eukaryotic ribosome biogenesis is a tightly regulated and coordinated process, and current data highlight a connection between ribosome maturation and the SUMO pathway. The connection was first established in *S. cerevisiae*, in which numerous SUMO pathway mutants, including a temperature-sensitive Ulp1, were shown to have defects in ribosomal RNA (rRNA) processing⁸⁷.

In mammalian cells, SENP3 has been found to physically interact with multiple proteins involved in ribosome maturation, including nucleophosmin (NPM1)^{88, 89} and a protein complex including PELP1, TEX10 and WDR18⁹⁰. The early steps of ribosome biogenesis occur in the nucleolus, and NPM1, SENP3 and SENP5 co-localize in subdomains of the nucleolus⁸⁹. Depletion of SENP3^{88, 89} or SENP5⁸⁹ results in rRNA processing defects, as does depletion of NPM1^{91, 92}. Although the substrates of SENP3 and SENP5 that are pertinent to ribosome biogenesis remain unclear, sumoylated forms of NPM1, GNL2 (a putative 60S preribosomal export factor) and the 60S ribosomal subunit RPL37A have been reported to accumulate upon depletion of SENP3, SENP5, or SENP3 and SENP5 in combination^{88, 89}. In the case of NPM1, the results of two reports are conflicting. While one study provides evidence that NPM1 is desumoylated by SENP3 and that constitutive sumoylation of NPM1 impairs rRNA processing⁸⁸, the other study presents data arguing against NPM1 as a substrate of SENP3 (or SENP5) but supporting a role for NPM1 in regulating the abundance of SENP3 and SENP5⁸⁹.

PELP1, TEX10 and WDR18 interact with SENP3 and one another⁹⁰. PELP1, TEX10 and WDR18 show distant homology to Rix1, Ipi1 and Ipi3, which form a complex involved in ribosome biogenesis in yeast^{93, 94}. PELP1 desumoylation by SENP3 is thought to control the localization of PELP1, which is partitioned between the nucleolus and the nucleoplasm⁹⁰. However, the importance of PELP1 localization can only be speculated at this time. Future studies on the roles of SUMO dynamics in ribosome biogenesis are expected to shed light on this exciting biological connection.

Mitochondrial dynamics

Even though most SUMO-regulated processes are localized to the nucleus, instances of SUMO functioning outside of the nucleus continue to emerge. One example is the control of mitochondrial dynamics^{95–97}. Many mitochondrial proteins are modified by SUMO1 in cultured mammalian cells, and SUMO1 overexpression leads to mitochondrial fragmentation⁹⁵. Concurrent overexpression of SENP5 can prevent this SUMO1-induced mitochondrial fragmentation and additional evidence supports roles for SENP5 in both mitochondrial fission and fusion⁹⁶. Although SENP5 localizes to the nucleus for most of the cell cycle, it is found at mitochondria during mitosis⁹⁷. SENP5 is required for cell

division^{46, 97} and the localization of SENP5 to mitochondria at mitosis is suggested to facilitate mitochondrial inheritance during cell division⁹⁷. Although SENP5 is expected to have many (unidentified) substrates on mitochondria, one established substrate is Drp1 (dynamin-related protein), a GTPase involved in mitochondrial fission⁹⁶. SUMO1 modification of Drp1 enhances its association with mitochondria, but subsequent desumoylation by SENP5 is thought to facilitate the assembly of Drp1 into active, fission-promoting polymers⁹⁷. However, the role of SENP5 in mitochondrial dynamics is not limited to regulating the function of Drp1 in fission since SENP5 knockdown also affects mitochondrial fusion and leads to a complex mitochondrial morphology that cannot be explained by the effects of SENP5 on Drp1 alone⁹⁶. Deciphering the exact roles of SUMO1 and SENP5 in mitochondrial dynamics will require the identification of more of the mitochondrial SUMO1/SENP5 substrates and further assays of mitochondrial fusion and fission.

SUMO proteases and the STUbL pathway

The surfaces of the SUMO and ubiquitin proteins differ markedly in their physical properties, and, unlike ubiquitin, SUMO does not directly target proteins to the proteasome. However, the recent discovery of a functionally conserved family of enzymes known as SUMO-targeted ubiquitin ligases (STUbLs), which catalyze the addition of ubiquitin to proteins that have been previously sumoylated, illuminated a pathway in which SUMO can indirectly target proteins for proteasome-mediated destruction or non-proteolytic but ubiquitin-dependent regulation^{98–101} [FIG. 5]. There are likely to be multiple STUbLs, but the best described are the related human RNF4 homodimer, the budding yeast Slx5–Slx8 heterodimer, and the fission yeast Rfp1/2–Slx8 heterodimer. These proteins all have multiple SIMs, and each subunit of the complex bears a RING domain that is important for dimerization and ubiquitin ligase activity^{102, 103}.

STUbL-mediated ubiquitylation is currently known to play key roles in regulating specific transcriptional programs¹⁰⁴ and DNA repair pathways^{105, 106}, processes also regulated by SUMO proteases (see above)¹⁰⁷. Since STUbLs and SUMO proteases can both affect cellular sumoylation dynamics, there are likely to be many interactions between these enzymes, and recent studies have indeed connected these two types of enzymes^{86, 100, 108, 109}. An outstanding example derives from a genetic screen using the *mot1-301* budding yeast mutant, which has a defect in the general transcriptional regulator Mot1¹⁰⁸. Genetic suppressors of the temperature-sensitive growth defect revealed mutations in *SLX5*, *SLX8*, SUMO E1, SUMO E2, or either of the two SUMO proteases (*ULP1* and *ULP2*). The mutant Mot1-301 protein is sumoylated, unlike the normal version of the protein, and loss of Slx5 or Slx8 leads to metabolic stabilization of the mutant protein, consistent with its potential STUbL-dependent ubiquitylation¹¹⁰. How mutations in *ULP1* and *ULP2* suppress the mutant *mot1-301* phenotype is less clear. Loss of SUMO protease activity might indirectly impair SUMO conjugation to Mot1-301 as a result of defects in precursor maturation and SUMO recycling.

Ulp1 and Ulp2 have been connected to Slx5–Slx8 in two other studies. Slx5 overexpression can partially compensate for loss of Ulp1, but only if mature SUMO is provided¹⁰⁰. These data suggest that although excess Slx5 cannot satisfy the SUMO precursor processing function of Ulp1, it can prevent growth defects caused by persistent or excessive sumoylation of certain proteins¹³. Enhanced Slx5–Slx8 STUbL activity may inactivate these proteins, either by targeting them to the proteasome for destruction or by simply removing them from their sites of action. Given what is known about STUbLs and SUMO proteases, one might reason that reduced STUbL activity would be exacerbated by reduced SUMO protease activity. However, defects associated with loss of Slx5–Slx8 are suppressed by

mutation of *ULP2*¹⁰⁹, suggesting that enhanced (poly)sumoylation of certain proteins can suppress STUbL deficiency. Since there are currently few known Ulp2 substrates and no confirmed natural substrates of Slx5–Slx8, these genetic interactions await molecular characterization. The genetic interaction between Ulp2 and Slx5–Slx8 is even more complex in that deletion of *SLX5* or *SLX8* suppresses the defects caused by lack of *ULP2*. Interestingly, an analogous situation has been observed in human cells, in that knockdown of RNF4 partially suppresses the mitotic defects caused by SENP6 knockdown (see above)⁸⁶.

Conclusions and perspectives

From recent work, Ulp and SENP enzymes are becoming better understood at the biochemical, cell biological and physiological levels. Many Ulp and SENP substrates have been identified and in some cases the mechanism by which desumoylation affects the activity or function of the target protein has been established. Still, each of the Ulp/SENP enzymes is likely to have many substrates, most of which await identification. Future studies are likely to be directed towards more global identification of specific SUMO protease substrates, including substrates of the newly identified DeSI enzymes and USPL1. Given that many sumoylated proteins are part of large macromolecular complexes, it will be important to determine how each SUMO protease substrate fits into networks of interacting proteins.

Sumoylation of many proteins greatly increases following certain cellular stresses, such as heat shock (see above), treatment with ethanol or hydrogen peroxide¹¹¹ and inositol starvation¹¹², but the direct involvement of SUMO proteases in these phenomena has been subject to only limited investigation. Notably, yeast cells exposed to high levels of alcohol, but not other tested stresses, relocalize Ulp1 to the nucleolus¹¹³. Whether nucleolar sequestration of Ulp1 is important for cells to survive high alcohol levels is not known. Identifying changes in SUMO protease activity, levels or localization in response to these stresses, in addition to isolating clusters of substrates that are differentially processed by these proteases, will be necessary to understand the physiological significance of these responses.

The catalytic domain of the Ulp and SENP proteases is confined to a ~200-residue stretch, but all of these enzymes are substantially larger. Besides their roles in cellular localization, the functions of the remaining domains are poorly defined. One study showed that a noncatalytic region of SENP2 is the most important region for SENP2 interaction with its substrate NEMO⁵⁶, suggesting that noncatalytic regions can play important roles in substrate recognition. Another study showed that the N-terminus of SENP2 influences the SUMO paralogue specificity of the enzyme, as full-length SENP2 displayed different activities than its catalytic domain⁴⁵. Most *in vitro* studies of Ulp and SENP enzymes have employed only catalytic domain fragments, and although challenging, more studies using full-length proteins are needed. The noncatalytic regions of Ulp and SENP enzymes may also bind accessory proteins that influence substrate specificity, activity and/or localization of the protease. Similarly, the noncatalytic regions of the DeSI enzymes and USPL1 are also likely to have unappreciated functions.

Many questions remain about STUbLs and their relationship to SUMO proteases. Does sumoylation of STUbL substrates have a function prior to their recognition and ubiquitylation by STUbLs? One attractive idea is that changes in the length of a substrate-linked SUMO chain can convert a protein for which sumoylation yields a particular functional activity into a STUbL substrate, triggering the end of the process that depended on the initial SUMO modification. Changes in SUMO protease activity could affect such a system. The existence of proteasome substrates that are modified by both SUMO and

ubiquitin raises several interesting questions [FIG. 5]. For example, does a SUMO protease act on these proteins before or during proteasome recognition of the substrate? Do any SUMO proteases physically associate with the proteasome? Although the site of ubiquitin conjugation on most STUbL substrates is unclear, there is evidence that STUbLs can add ubiquitin directly to the SUMO or SUMO chain moieties of these substrates^{114, 115}. Processing of such substrates might involve a protease that cleaves between SUMO and ubiquitin, and such an activity has been suggested for the yeast protein Wss1, a metalloprotease that has been genetically linked to both the SUMO and STUbL pathways^{115, 116}. We can expect that many of these central questions will begin to be answered over the next few years.

Acknowledgments

We thank Christian Schlieker and Jennifer Gillies for their helpful comments on the manuscript. We also acknowledge support from NIH grants GM046904 and GM053756 to M.H., an NIH NRSA postdoctoral fellowship (F32 GM097794) to C.M.H., and an NSF predoctoral fellowship to N.R.W.

Glossary

nuclear pore complex	Large multiprotein complex that forms a channel in the nuclear envelope of an eukaryotic cell, joining the inner and outer nuclear membranes and allowing transport of proteins to and from the nucleus.
Septin	Highly conserved protein family first identified in yeast and more recently found in a wide range of animal cells. They are thought to function primarily in the control of cytokinesis in yeast, where they form a 10-nm filamentous ring that encircles the bud neck.
ubiquitin-proteasome system	A system of selective, ATP-dependent protein degradation, in which target proteins that have been conjugated by ubiquitin are degraded by the 26S proteasome.
histone deacetylase	An enzyme that removes the acetyl groups of core histones; its activity has an important function in transcriptional regulation and cell-cycle progression through alterations in chromatin structure.
polycomb-related repressors	A family of proteins that can remodel chromatin and silence genes. This protein family was first discovered in <i>Drosophila melanogaster</i> .
homologous recombination	A DNA recombination pathway, which includes the repair of double-strand DNA breaks, that uses a homologous double-stranded DNA molecule as a template for the repair of the broken DNA.
Centromeres	Region of a chromosome that is attached to the spindle during nuclear division.
mitochondrial fission	Mitochondrial membrane constriction and scission that promotes fragmentation of the mitochondrial network. The process is highly regulated and together with the opposing process of mitochondrial fusion (joining) is responsible for the dynamics observed for the mitochondrial network.
response element	A short sequence of DNA within the promoter region of a gene that binds specific DNA-binding transcription factors, thus impacting transcription of the (typically adjacent) gene.

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Box 1 | SUMO-interacting motifs

A SUMO-interacting motif (SIM) is a short sequence element (less than 10 amino acids), in some cases including phosphorylated amino acids, that is found in many proteins and that interacts with a specific surface groove of SUMO¹⁰. Although the properties of all SIMs are conserved, consensus sequences for given SUMO paralogue-SIM interactions are only beginning to emerge¹¹⁷. Most SIMs that have been identified to date have two important elements, a core of 3–4 hydrophobic residues (usually valine or isoleucine) and a nearby acidic region, which can be glutamate or aspartate side chains or phosphorylated serines or threonines. The hydrophobic residues of the SIM bind to the β 2 strand of SUMO and extend the β -sheet of the β -grasp fold; the acidic residues bind a nearby basic patch on the SUMO surface, reinforcing the interaction and orientation of the SIM (parallel or anti-parallel to the β 2 strand)^{118, 119}. Although the SIM is the best-studied motif that binds SUMO, a variant SUMO-binding motif has recently been described¹²⁰, and others are likely to be discovered.

Figure 1. SUMO conjugation and deconjugation cycle

a | SUMO (S) is translated in a precursor form (black) that must be processed by a SUMO protease before SUMO can be conjugated to other proteins. Mature SUMO is conjugated to proteins in an ATP-dependent manner in reactions catalyzed by three enzymes, E1, E2, and E3, in sequence. SUMO can be conjugated to a single lysine of a protein (monosumoylation), multiple lysines of a single protein (multisumoylation), or to SUMO that is already conjugated to a protein, forming a SUMO chain (polysumoylation). SUMO proteases deconjugate SUMO or SUMO chains from proteins. See Table 1 for a summary of the ability of each of the known SUMO protease to process and/or deconjugate the different forms of SUMO. **b** | The C-termini of *S. cerevisiae* Smt3 and the four mammalian SUMO isoforms are shown, with the di-glycine motif underlined. The site of cleavage is indicated by an arrow. The chemical structure of the C-terminal tail of SUMO2 is also shown, with the scissile bond boxed in orange (bottom). **c** | The chemical structure of the isopeptide bond between the terminal glycine of mature SUMO and the lysine of a substrate is also shown, with the scissile bond boxed in orange.

Figure 2. Structures of SUMO proteases

The domain organizations and lengths (in amino acids) of the SUMO proteases discussed in this article are shown. Each catalytic domain (CD) is colored magenta, with key histidine (H) and cysteine (C) residues shown. Putative SUMO-interacting motifs (SIMs) are indicated by asterisks. Regions shown to be important for intracellular localization of the protease are shown in cyan. For DeSI-1 and DeSI-2, the mouse proteins are shown. For USPL1, the human protein is depicted. Representatives from each of the three known classes of SUMO proteases exist in other organisms^{15, 21}; however, in some cases it is unclear whether these proteins are indeed SUMO-specific proteases. **b** | Crystal structure of the catalytic domain of SENP1 (magenta) in complex with SUMO2 (blue) (Protein Databank (PDB) code 2CKH) shows the orientation of SUMO2 with respect to SENP1. This complex contains a covalent thiohemiacetal linkage between the active site cysteine of SENP1 and the C-terminal glycine of SUMO2. The catalytic residues are shown in stick format, with the atoms colored according to element (carbon = cyan; nitrogen = blue; oxygen = red; sulfur = orange). **c** | A space-filling representation of the structure shown in part b, which highlights the intimate contacts made between SENP1 and SUMO2. **d** | A higher magnification view of

SEN1 in complex with SUMO2 (2CKH), showing the two Trp residues of SEN1 that surround the two Gly residues of SUMO2. **e** | The structure of the catalytic domain of SEN7 (yellow) (PDB code 3EAY) aligned to the structure of the catalytic domain of SEN1 (magenta) highlights the similarities and differences between SEN1/2 and SEN6/7. The catalytic residues are displayed in cyan for SEN1 and red for SEN7. Loop regions, including Loop-1 (*right*, green), of SEN7 are unique to SEN6 and SEN7. **f** | The structure of a monomer of DeSI-1 (PDB code 2WP7) shows the proposed catalytic dyad (Cys-His), which differs from the catalytic triad (Cys-His-Asp) utilized by the Ulp/SEN1 enzymes. The catalytic residues are displayed as in **b**

Figure 3. Regulation of SUMO proteases

a | SENP1 and SENP2 levels are known to be affected by changes in their transcription under various conditions (see text). Fittingly, the promoters of SENP1 and SENP2 contain response elements (REs) that bind specific transcription factors (TFs). Both SENP1 and SENP2 can affect their own transcription by desumoylating transcription factors or factors that affect the activity or abundance of relevant transcription factors. **b** | SENP3 levels are regulated by the ubiquitin-proteasome system. SENP3 is constitutively ubiquitinated under normal growth conditions, leading to its proteasome-mediated degradation. Under conditions of mild oxidative stress, SENP3 ubiquitination is reduced and SENP3 levels increase. When the tumor suppressor p19(Arf) is expressed, it interacts with SENP3, enhancing its ubiquitination and degradation, leading to decreased levels of SENP3. **c** | Yeast Ulp2 is phosphorylated during the mitosis phase of the cell cycle. Phosphorylation of Ulp2 is thought to inhibit its activity.

Figure 4. The function of SENP1 in transcription during Wnt signaling

Activation of Wnt signaling leads to the sumoylation of TBL1 and TBLR1, which function together as a transcriptional co-activator complex. Sumoylation of TBL1-TBLR1 promotes the dissociation of TBL1-TBLR1 from the NcoR/HDAC3 corepressor complex and promotes the association of TBL1-TBLR1 with β-catenin. The TBL1^{SUMO}-TBLR1^{SUMO}-β-catenin complex is recruited to Wnt target genes by the sequence-specific DNA-binding protein TCF4. SENP1 is responsible for desumoylation of TBL1^{SUMO}-TBLR1^{SUMO}, which leads to the disassembly of the TBL1^{SUMO}-TBLR1^{SUMO}-β-catenin complex and decreased activation of Wnt target gene expression.

Figure 5. SUMO-targeted ubiquitin ligases (STUbLs)

STUbLs are enzymes that catalyze the addition of ubiquitin to proteins that have been previously sumoylated. SUMO (S), likely in the form of SUMO chains, attached to STUbL substrates is recognized by SIMs of the STUbL. The ubiquitin (U)-charged E2 enzyme (E2-U) is recruited by the dimeric RING domains of the STUbL, and ubiquitin is transferred to the substrate. Potential sites of attachment for ubiquitin on the substrate include the substrate itself and/or the attached SUMO moieties. STUbL activity results in proteins that are modified by both SUMO and ubiquitin. Ubiquitin addition can target proteins to the proteasome for destruction or non-proteolytic fates. It is currently unclear whether SUMO proteases act on STUbL substrates destined for the proteasome. If SUMO proteases act on these substrates, it could be before arrival at the proteasome (1) and/or at the proteasome (2). Alternatively, SUMO could be destroyed along with the substrate at the proteasome. Ubiquitin on STUbL substrates is likely recycled by deubiquitylating (DUB) activity of the proteasome (not shown).

Table 1

Properties of the SUMO proteases.

Species	Name	Subcellular Localization	SUMO Isoform Preference	Precursor processing	Deconjugation	Chain Editing**
<i>S. cerevisiae</i>	Ulp1	nuclear pore	Smt3	Yes	Yes	No
	Ulp2	nucleoplasm	Smt3	No	Yes	Yes
Mammals	SEN1	nuclear pore and nuclear foci	SUMO1 and SUMO2/3	Yes	Yes	No
	SEN2*	nuclear pore and nuclear foci, cytoplasm	SUMO2/3 > SUMO1	Yes	Yes	No
	SEN3	nucleolus	SUMO2/3	Unknown	Yes	No
	SEN5	nucleolus and mitochondria	SUMO2/3	Yes	Yes	No
	SEN6	nucleoplasm	SUMO2/3	No	Yes	Yes
	SEN7	nucleoplasm	SUMO2/3	No	Yes	Yes
	DeSI-1	cytoplasm and nucleus	SUMO1 and SUMO2/3	Weak	Yes	Yes
	DeSI-2	cytoplasm	Unknown	No	Unknown	Unknown
	USPL1	Cajal bodies	SUMO2/3 > SUMO1	Weak	Yes	Yes

* SEN2 has at least 3 splice variants that localize differentially in cells. The 589 amino acid version localizes to the nuclear pore.

** Chain editing refers to the ability of a protease to remove SUMO moieties from a SUMO chain without removing the entire SUMO chain from sumo-modified proteins.