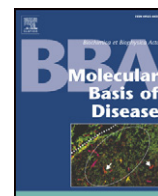Contents lists available at ScienceDirect

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Review

Emerging roles of desumoylating enzymes

Jung Hwa Kim [a],*, Sung Hee Baek [b],*

[a] Department of Medical Sciences, Inha University, Incheon, South Korea
[b] Department of Biological Sciences, Cancer Research Institute, Seoul National University, Seoul, South Korea

A R T I C L E   I N F O

A B S T R A C T

Posttranslational modification by small ubiquitin-like modifier (SUMO) controls diverse cellular processes including transcriptional regulation, nuclear transport, cell-cycle progression, DNA repair, and signal transduction pathway. Sumoylation is a highly dynamic process that is reversed by a family of Sentrin/SUMO-specific proteases (SENPs). Thus, desumoylation process must be important for regulation of the fate and function of SUMO-conjugated proteins as well as SUMOylation process. SENPs catalyze the removal of SUMO from SUMO-conjugated target proteins as well as the cleavage of SUMO from its precursor proteins. Since the first report of yeast desumoylating enzymes, many studies have revealed the structural and cellular biological properties of SENP family. This review focuses on the specificity of the SENPs' catalytic activities with regard to SUMO isoforms and their emerging roles as cellular regulators.

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1. Introduction

Ubiquitin (Ub) and ubiquitin-like proteins (Ulps) are covalently conjugated to target proteins and alter the properties of modified proteins [1,2]. SUMO has about 18% identity to Ub in amino acid sequence and executes a variety of biological functions comparable to Ub [3–5]; it is involved in the control of gene expression, genomic and chromosomal integrity, intracellular transport, and protein stability [6]. The list of proteins subjected to sumoylation is rapidly growing, and the event of sumoylation is not confined to the nucleus but occurred in most subcellular compartments [7,8].

SUMO conjugation to target proteins results in the formation of isopeptide (amide) bond between the C-terminal glycine of SUMO and the ε-amino group of lysine within the target proteins. Similar to ubiquitination, sumoylation is a dynamic process that can be readily reversed. Therefore, cellular abundance of particular SUMO-conjugated substrates is regulated by a balance between SUMO conjugation and SUMO deconjugation. Yeast enzymes responsible for SUMO processing (C-terminal hydrolase activity) and deconjugation (isopeptidase activity) are referred to as Ubl-specific proteases (Ulp) [9,10]; the Ulp found in humans are referred to as SENPs [11–13].

During SUMO metabolism, Ulp/SENPs catalyze three distinct processes: processing, deconjugation, and chain editing [14]. Like ubiquitin, SUMO proteins are expressed as precursor proteins that carry a C-terminal extension of variable length (2–11 amino acids) found after a conserved di-glycine motif. To function as a modifier of

target proteins, the C-terminal di-glycine motif of the SUMO proteins must be exposed by the action of SUMO specific protease [2]. SUMO processing activity of SENPs is responsible for cleavage after the C-terminal di-glycine motif and SUMO deconjugating activity of SENPs is required for the cleavage of amide bond between the C-terminus of the mature SUMO and the ε-amino group of the target lysine within the substrates. Chain editing refers to the dismantling of SUMO chains.

This review summarizes the recent findings of substrate specificity of SENPs that demonstrate selectivity for the SUMO substrates and the current knowledge of the biological functions of desumoylating enzymes as diverse cellular regulators.

2. The SUMO family and SUMO pathway

The budding yeast *Saccharomyces cerevisiae* expresses one SUMO ortholog named Smt3 [15]. In mammals, three SUMO family members, designated SUMO-1, SUMO-2 and SUMO-3 are expressed [16]. The mature forms of SUMO-2 and SUMO-3 share 97% identity, but have only a 50% sequence identity with SUMO-1 [1,2,17]. The mature forms of SUMO-2 and SUMO-3 only differ from one another by three amino-terminal residues; these two isoforms have sometimes been referred to interchangeably in the literature. Here, we will refer to the isoform with two amino acids after the di-glycine motif as SUMO-2 (Gly-Gly-Val-Tyr) and the one with 11 amino acids as SUMO-3 (Gly-Gly-Val-Pro-Ser-Ser-Leu-Ala-Gly-His-Ser-Phe). A fourth member of the human SUMO protein family (SUMO-4) has a predicted 86% amino acid similarity with SUMO-2, and it has been reported to be expressed mainly in the kidneys, lymph nodes, and spleen [18]. However, Proline 90 in SUMO-4 appears to inhibit the maturation of precursor of SUMO-4 and it is unclear whether SUMO-4 is covalently modified to target substrates *in vivo* [19].

* Corresponding authors. S.H. Baek is to be contacted at tel.: +82 2 880 9078; fax: +82 2 886 9078. J.H. Kim, tel.: +82 32 860 8192; fax: +82 32 876 8077.
E-mail addresses: jhkim4@inha.ac.kr (J.H. Kim), sbaek@snu.ac.kr (S.H. Baek).

Prior to conjugation, mature SUMO, which has an exposed C-terminal di-glycine motif, is activated by the SUMO-activating enzyme (E1), a heterodimeric protein composed of Uba2 and Aos1 [20–23]. SUMO is then transferred to the catalytic Cys residue of Ubc9, a conjugation enzyme (E2) [24–27]. Finally, an isopeptide bond is formed between the C-terminal Gly residue of SUMO and the ε-amino group of a lysine residue in the target protein. At this step, SUMO protein ligase (E3) helps the modification to be more efficient. An intriguing feature of SUMO modification is that target lysine residues are usually located in a consensus SUMO modification motif, ΨKxE/D (where Ψ is a large hydrophobic residue and x is any amino acid) [2,6,28]. This is remarkable because such a sequence is lacking in the ubiquitin system. Ubc9 is capable of recognizing this motif and transferring SUMO moiety to all SUMO substrates [4,28]. However, some SUMO substrates lacking this motif have been identified [29,30]. Maybe SUMO E3 ligases facilitate and increase SUMO modification of proteins lacking this motif. Several SUMO E3 enzymes have been identified, and the PIAS family proteins contain a modified RING domain and bind SUMO non-covalently [31–36]. The polycomb protein Pc2 and the nucleoporin RanBP2 also act as SUMO E3 ligases [37–40].

There are several noticeable distinctions between SUMO-1 and SUMO-2/3. One obvious difference is the presence of a large pool of free or non-conjugated SUMO-2/3 compared to SUMO-1 in the cell. The existing nonconjugated pool of SUMO-2/3 can be readily conjugated to high molecular mass proteins in reaction to stress stimuli such as heat shock, oxidative stress, and ethanol exposure [41]. In addition, individual target proteins can be selectively modified by SUMO-1 and SUMO-2/3. For example, the Ran GTPase-activating protein (RanGAP) 1, is preferentially modified by SUMO-1, but very poorly modified by SUMO-2/3 [41]. Interestingly, SUMO-2 and SUMO-3 have consensus SUMO modification motif in the N-terminal region (Lys11), but SUMO-1 does not [11,12,42]. An internal sumoylation site is also found in Smt3 (Lys15). Thus, analogous to Ub conjugation, SUMO-2 and SUMO-3, as well as the yeast Smt3, can form polymeric SUMO chains [42,43]. Poly-SUMO chains are detected in *S. cerevisiae*; however, SUMO chain formation has been shown not to be a major feature of SUMO function in yeast, either for production of the most abundant conjugates or for the activities required for yeast viability and stress resistance [44].

In higher eukaryotes, the importance of poly-SUMO chains for the regulation of target proteins has been suggested. Elimination of SUMO-3 protein by siRNA causes destabilization of promyelocytic leukaemia (PML) localization in the nucleus, indicating a crucial role for poly SUMO-3 chain formation in the nuclear body (NB) dynamics [45]. In contrast to ubiquitination, SUMO modification does not usually trigger protein degradation; the action of SUMO as an inhibitor of ubiquitin-mediated degradation is well documented. IκBα, Smad4, Huntingtin, PCNA, Rad52, HIF-1α and Mdm2 are well-known examples of substrates that escape from degradation by the protection of SUMO [7,46–53]. However, the results of recent studies in yeast and in human cells have suggested that polymeric SUMO chains (polysumoylation) play a role in the promotion of ubiquitin-mediated proteasomal targeting [54,55]. The first identified protein degraded by polysumoylation-dependent polyubiquitination was reported to be PML. Increase of polymeric SUMO-2 modification of PML by arsenic trioxide (ATO, As₂O₃) treatment has been shown to lead to its subsequent polyubiquitination by RNF4, ubiquitin E3 ligase followed by efficient degradation [56,57]. Polysumoylation of BMAL1 with the SUMO-2/3 moiety serves as a targeting signal for its ubiquitination and proteasomal degradation *in vivo* [58]. It appears that, as in the case of ubiquitin, monomeric and polymeric chains of SUMO proteins have distinct biological functions.

3. Desumoylating enzymes — Ulp/SENPs

In budding yeast, two Smt3-specific proteases, Ulp1 and Ulp2, have been characterized [9,10]. The similarity between Ulp1 and Ulp2 has been confined primarily to a protease domain of ~200 amino acids
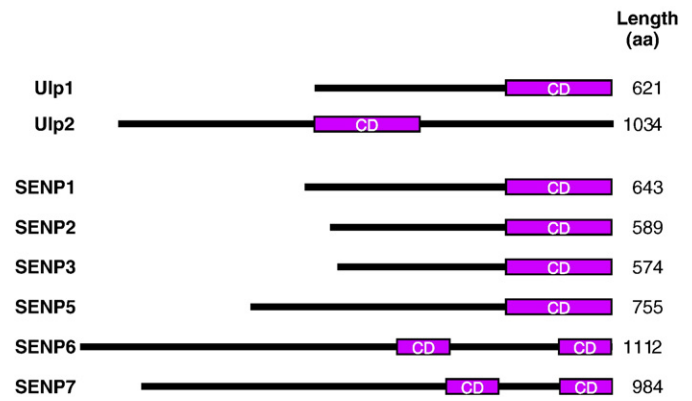
Fig. 1. Primary structure of Ulp/SENPs. The conserved domain is shown in the purple box. The black bars represent the nonconserved variable regions. Note that the catalytic domains of SENP6 and SENP7 are divided by the insertions.

referred to as the Ulp domain (UD) [9]. Database searches initially identified eight genes for human proteins with significant sequence similarity to the C-terminal Ulp domain of yeast Ulp1 that were believed to be SUMO-specific proteases (SENP1–8) (Fig. 1) [59]. Later, SENP3 and SENP4 turned out to be the same proteins. Not all SENPs are SUMO-specific; indeed SENP8 (also called NEDP1 or DEN1) has specificity toward another small ubiquitin-like protein, NEDD8 [60,61]. At present, six true human SENPs can be divided into two groups. SENP1, SENP2, SENP3, and SENP5 are most closely related in sequence to Ulp1, whereas SENP6 and SENP7 are related to Ulp2 [11,62]. SENP6 and SENP7 contain an additional insertion that splits the conserved catalytic domain, but the function of this inserted sequence remains to be identified [12,63].

Ulp/SENPs are cysteine proteases that contain a conserved C-terminal domain with the characteristic His-Asp-Cys catalytic triad. These enzymes belong to the clan CE proteases and their mechanistic relatives, deubiquitinating enzymes (DUBs), are classified to the clan CA of papain-like proteases [64]. From the viewpoint of the closely related sequences between the Ub and SUMO conjugation pathway enzymes (E1 and E2), it is surprising to find that there are no sequence similarities between Ub and SUMO deconjugating enzymes [64]. There have been developed many biochemical assays to determine the UBL specificities of the UBL isopeptidases [65–69]. Those studies will be very helpful for characterizing a novel modulator of isopeptidase activity and screening useful drugs for UBL isopeptidase-related diseases.

The non-conserved N-terminal regions of the Ulp/SENPs are thought to determine the subcellular localization and substrate specificity of each protease. Ulp1 has been detected at the nucleoplasmic face of the nuclear pore complex (NPC) [9,70,71], and Ulp2 is present in the nucleoplasm [9]. The non-conserved N-terminal domain of Ulp1 is necessary for the correct localization of Ulp1 at the nuclear envelope [70]. Remarkably, N-terminal deleted Ulp1, but not full-length Ulp1, suppresses the cellular defects of *ulp2Δ* mutant cells and substantially reduces Smt3-protein conjugates. The non-catalytic N-terminal domain of Ulp1 functions as a physiologically significant restraints with regard to the subset of sumoylated proteins which are natural targets of nucleoplasmic Ulp2. Subcellular localization of Ulp/SENPs is, at least in part, an important constraint of SUMO isopeptidase specificity [70].

SENP1 contains both N-terminal localization signals (NLS) and nuclear export signals (NES), but the primary location of SENP1 is in the nucleus [13,72,73]. In CV-1 cells, GFP-SENP1 was localized mainly in the cytoplasm but relocalized into the nucleus after Leptomycin B (LMB) treatment, indicating the nucleo-cytoplasmic shuttling of SENP1. Upon HIPK2 co-expression, GFP-SENP1 was translocated into
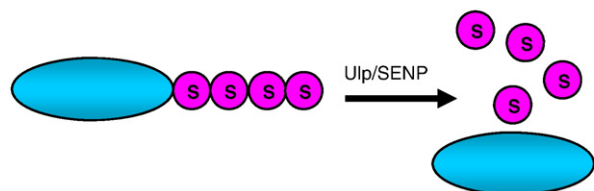
A Processing of SUMO precursors
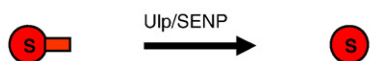
B Deconjugation of SUMO conjugation
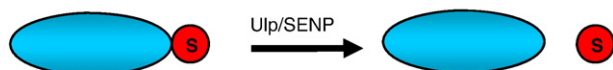
C Chain editing of SUMO chains

Fig. 2. Catalytic functions of desumoylating enzymes. (A) Processing of SUMO precursors by Ulp/SENPs generate free SUMO monomers that are ready for protein modification. (B) Reversal of SUMO conjugation occurs by deconjugation of SUMO from modified proteins. (C) Ulp/SENPs function in the editing of SUMO at the distal ends of poly-SUMO-2/3 chains conjugated to protein substrates. The red circle represents SUMOs and the pink circle represents SUMO-2/3.

the nucleus [74]. Probably, SENP1 localization might be controlled by the extracellular signaling pathways with concurrent post-translational modifications such as sumoylation or phosphorylation, though any data that can prove the hypothesis has not yet been reported [74]. SENP2 is closely related to SENP1; it is primarily found in the nuclear envelope in association with the NPC [75,76]; it also accumulates in distinct subnuclear bodies [77]. It has been reported that SENP2 has both NLS and NES within its non-conserved N-terminus. Using a series of SENP2 mutants and interspecies heterokaryon assay, it has been shown that SENP2 shuttles between the nucleus and the cytoplasm and that the shuttling is blocked by mutations in the NES or by LMB treatment. Interestingly, the SENP2 in the cytoplasm is efficiently polyubiquitinated and triggered to be degraded by the 26S proteasome pathway [78]. In addition, alternative mRNA splicing generates at least three different SENP2 isoforms with strikingly distinct cellular localization (Axam, SMT3IP2/Axam2, SuPr-1). Axam localizes to the nucleoplasmic face of the NPC [76], and Axam2 and SuPr-1 have been detected in the cytosol and PML bodies [79,80]. This pattern of isoform localization underscores the notion that a distinct subcellular distribution might contribute to the substrate specificity of the Ulp/ SENPs. SENP3 and SENP5 are detected predominantly in the nucleolus [81,82]. Very recently, it has been reported that SENP3 is destabilized in a nucleolar protein nucleophosmin (NPM1)-dependent manner by a process involving sequential p19^Arf-induced SENP3 phosphorylation, polyubiquitination and raid proteasomal degradation [83]. SENP6 was originally reported to be located in the cytoplasm [84], but recent reports suggest that it is located in the nucleus [62,85]. Recently identified mouse SUSP4 has been found to be closely related to human SENP2, and was detected predominantly in the nuclear fractions. SUSP4 has been shown to be ubiquitously expressed, at varying levels, in different tissues and cell lines [48].

4. Catalytic mechanism of desumoylating enzymes

There have been many structural and biochemical investigations of Ulp/SENPs to identify their specificity for SUMO isoforms, in addition to the differentiation among processing, deconjugation and chain editing reactions (Fig. 2 and Table 1). Crystal structures of several Ulp/SENPs have revealed that these proteases have narrow and tight active site tunnels for di-glycine motif recognition and utilize conserved tryptophans to clamp down on the C-terminal di-glycine motif of SUMO within a hydrophobic channel [5,86,87]. Clan CA protease DUBs also contain a restricted tunnel for the two Gly residues at the C-terminus of ubiquitin [88,89]. And each family contains size dominant exosite that binds epitopes on the Ub or SUMO surface, respectively. By substrate positional scanning profile Drag et al. determined the optimal cleavage motif for SENPs (except SENP3) and several DUBs [14,90]. Not surprisingly, SENP1, 2, and 5 showed optimal preferences for the SUMO C-terminal tetrapeptide sequence QTGG and DUBs for the LRGG sequence corresponding to the Ubiquitin C-terminus. Surprisingly, SENP6 and 7 prefer the LRGG sequence. However, none of the known LRGG-containing natural substrates was processed by SENP6 and 7. Interestingly, in contrast to the wide conformation changes and catalytic site reordering of DUBs upon Ub binding, catalytic domains of desumoylating enzymes did not undergoes large conformational changes for complex formation with SUMO [64,87].

Ulp1 and Ulp2 have distinct and non-redundant catalytic activity. A large amount of mature Smt3 weakly rescues the lethal course of the $ulp1\Delta$ mutant cells, but full-length Smt3 does not [10]; these findings suggest that Ulp1 plays an important role in Smt3 processing. By contrast, it appears that Ulp2 is specialized for chain editing activities. In yeast, mutants lacking Ulp2 accumulated high molecular weight SUMO-containing species only in the presence of wild-type Smt3; however, this was not the case in the $smt3$ mutants that did not have the critical lysine residues [44]. Further, Smt3 branch-site mutants suppressed several phenotypes of $ulp2\Delta$, indicating that such defects arise from Smt3-chain accumulation [44].

Recently, structures have been determined for the catalytically inert form of SENP1 and the SENP2 catalytic domain, in complexes with actual substrate-SUMO precursors and SUMO-modified Ran-GAP1 [91,92]. In both cases, the scissile peptide bond is kinked at a right angle to the C-terminal tail of SUMO-1 due to a cis arrangement of the amide nitrogens. As a result of this structure, it is cleared that a kinked, cis configuration for the scissile bond is crucial for the cleavage of both peptide and isopeptide bonds. SENP1 has both C-terminal hydrolase and isopeptidase activities and, although it does not discriminate among SUMO isoforms during deconjugation, it is strongly isoform specific during SUMO protein processing [86,93]. SENP1 processes SUMO-1 in preference to SUMO-2, but shows limited activity with regard to SUMO-3. The histidine residue immediately after the di-glycine motif of SUMO-1 confers rapid processing by SENP1 $in vitro$ and Pro94 in SUMO-3 is responsible for the resultant slow processing [93]. The K_m values for SUMO-1 and SUMO-3 cleavage

Table 1
Ulp/SENPs localization and substrate specificity of catalytic activity

Name	Subcellular localization	Processing	Deconjugation	Chain editing
Saccharomyces cerevisiae				
Ulp1	Nuclear periphery	Yes	Yes	No
Ulp2	Nucleoplasm	No	No	Yes
Humans				
SENP1	Nuclear speckled foci, nuclear pore	SUMO-1>SUMO-2>SUMO-3	SUMO-1/2/3	No
SENP2	Nuclear pore	SUMO-2>SUMO-1>SUMO-3	SUMO-1/2/3	No
SENP3	Nucleolus	Unknown	SUMO-2/3	No
SENP5	Nucleolus	SUMO-3	SUMO-2/3	No
SENP6	Nucleoplasm	No (very low)	SUMO-1	SUMO-2/3
SENP7	Nucleoplasm	No (very low)	No (very low)	SUMO-2/3

are very similar, but the catalytic constant (k_{cat}) for SUMO-3 is 50-fold lower than that for SUMO-1. Therefore, the ability of SENP1 to differentially cleave SUMO-1 over SUMO-3 is not a consequence of preferential binding of substrate, but rather is based on a more efficient catalytic process [91]. This may result from the differences in the ability of SENP1 to kink the scissile bonds in SUMO-1 and SUMO-3. In the *cis* amide conformation, the side chain of His98 of SUMO-1 forms a hydrogen bond to the Gly600 of SENP1. However, the proline residue in SUMO-3 is likely to have an inhibitory effect on the orientation of the scissile peptide bond due to its rigid structure [91].

SENP2 can deconjugate RanGAP1-SUMO-1, RanGAP1-SUMO-2 and RanGAP1-SUMO-3 with similar efficiency, but shows different substrate preferences during processing. SENP2 processes SUMO-2 more efficiently than SUMO-1, but processes SUMO-3 poorly as with SENP1 [87]. The SENP2 structures reported by Reverter et al., showed that in both productive processing (SENP2-pre-SUMO-3) and deconjugation (SENP2-RanGAP1-SUMO) complexes, the scissile peptide bond is coordinated by a protease hydrogen bond network formed by the SENP2 Val477 and Gln542 side chain, which stabilizes a 90° kink in the substrate, directing residues on the C-terminal side of the scissile bond toward a protease surface composed of SENP2 Trp420, Val477, Gly545 and Met474. This geometry appears to be unfavourable for the processing SUMO precursors; the side chains on the C-terminal side of the cleavage site present a steric impediment for interaction with the SENP2 surface. This explains why SENP2 acts more efficiently in deconjugation than in processing [92].

SENP5 displays both C-terminal hydrolase and isopeptidase activities. SENP5 shows SUMO-3 C-terminal hydrolase activity, but does not process SUMO-1 *in vitro*. In addition, SENP5 demonstrates isopeptidase activity with SUMO-2 and SUMO-3 conjugates but not against SUMO-1 conjugates *in vivo* [82,94]. SENP3 displays specificity for SUMO-2 and SUMO-3 conjugates, but not for SUMO-1 [82,95]. SENP6 has a special role in the dismantling of highly conjugated SUMO-2 and SUMO-3. SiRNA-mediated depletion of SENP6 suggests that it might function to accurately maintain nuclear structures, particularly PML bodies [62]. In addition, it has also been reported that SENP6 has isopeptidase activity with regard to a single SUMO moiety. SENP6 specifically removes SUMO-1 from retinoid X receptor (RXR)α, but not from androgen receptor (AR) or peroxisome proliferator-activated receptor (PPAR)γ [85]. Recently, the structure of the SENP7 catalytic domain (662 to 984) has been characterized by Lima and Reverter [95]. Using biochemical assays they demonstrated that SENP6 and SENP7 have excellent deconjugating activity for poly-SUMO-2/3 chains. Structural analysis has shown that SENP7 catalytic domain contains four Loops unique to the SENP6/7 subfamily of Ulp/SENP family members. Val713 is positioned in SENP6 and SENP7 immediately adjacent to Loop-1, whereas this residue was substituted by glutamate in SENP1 and SENP2. Removal of these features by Loop-1 deletion or Glu substitution for Val diminishes SENP7 activity with regard to poly SUMO-2/3 chains. These unique determinants within the SENP6/7 subfamily appear to contribute to the deconjugation of poly-SUMO chains [95]. According to the activity profiling of human SENPs using recombinant catalytic domains of SENPs (SENP3 is omitted owing to difficulties in obtaining sufficient soluble protein), SENP1 was found to be the most efficient C-terminal hydrolase, but it has substantially higher isopeptidase activity compared to C-terminal hydrolase activity [96]. This suggests that SENP1 might be the most active SUMO hydrolase *in vivo*.

5. Desumoylating enzymes in plants and parasitic protozoa

A database search, as well as genetic and biochemical analyses, revealed that components of the SUMO conjugation and deconjugation systems are conserved in plants such as *Arabidopsis*, rice, tomato, and *Medicago* [97]. In plants, sumoylation has been implicated in phosphate starvation responses, cold tolerance, basal thermotoler-

ance, pathogen defence, and regulation of flowering time. To date, four *Arabidopsis* SUMO proteases have been characterized functionally [98]. All of them have SUMO precursor protein processing and substrate deconjugation activity. Significantly, they display SUMO isoform specificity and discriminate between processing and deconjugation.

By literature and BLASTP homology searches Ponder and Bogyo made a comprehensive list of homologs of known Ubls and Ubl-deconjugating enzymes in medically important parasitic protozoa [99]. Similar to yeast, most of the organisms examined have a single SUMO homolog. And of the parasites surveyed, one or two homologs of the essential yeast desumoylating enzyme Ulp1 were found.

6. Role of desumoylating enzymes in biological pathways

Desumoylating enzymes are involved in the regulation of SUMO-dependent pathways by balancing the sumoylation status of target proteins. Ulp/SENPs participate in diverse biological pathways including transcriptional regulation, development, cell growth and differentiation, cancer, and ribosome biogenesis.

6.1. Transcriptional regulation

More than 120 mammalian SUMO substrate proteins have been identified. Two thirds of them are transcription factors and co-regulators [7,100]. In some cases, SUMO modification enhances the activity of transcription activators and co-activators; however, the majority of this modification is biased towards the enhancement of the inhibitory effects on transcription by the transcription factors and co-regulators. Therefore, desumoylation activity of Ulp/SENPs plays an important role in the derepression of the inhibited transcription by SUMO modification. SENP1 relieves the SUMO-dependent repression of AR, p300, Ets1, Reptin, and KAP1 [101–105]. Enhancement of AR-dependent transcription is not mediated by desumoylation of AR, but rather is processed by the deconjugation of histone deacetylase 1 (HDAC1), thereby reducing its deacetylase activity [104]. The action of SENP1, on *c-Jun* transcription, is mediated by the derepression of p300 by SUMO removal [105]. Desumoylation of Reptin by SENP1 significantly attenuates binding to HDAC1 and induces the expression of the metastasis suppressor gene *KAI1* [102]. SUMO removal of KAP1 by SENP1 increases the basal phosphorylation of KAP1 on Serine 824 site, which stimulates transcription of p21 and Gadd45α [101]. SUMO cleavage by SENP2 (SuPr-1) potentiates transcriptional activation of Sp3 and changes the subcellular localization of Sp3 [77]. Although SENP2 has been isolated as a c-Jun activator, c-Jun is not a direct target of SENP2. Instead, PML plays a major role in the SENP2 action on gene transcription. Coactivators kept in the PODs by PML were reported to be released by SENP2 binding to SUMO-conjugated PML [80]. SMT3IP2/Axam2, the mouse homolog of SENP2, negatively regulates the Wnt signaling pathway by promoting the degradation of β-catenin. However, this function of Axam2 is independent of its protease activity, and the involvement of Axam2 in Wnt signaling remains to be further elucidated [79]. MEF2, a transcriptional activator of muscle differentiation, is also a SUMO target. SENP3 reverses the modification to augment the transcriptional and myogenic activities of MEF2 [106]. The isopeptidase activity of SENP6 acts on RXRα, but not on AR and PPARγ, and increases the RXR transcriptional activity [85].

6.2. Development

The characterization of a random retroviral insertional mutation in the mouse ortholog of SENP1 highlights the critical importance of regulating the levels of sumoylation during mouse embryogenesis, which suggests a non-redundant and essential role for SENPs during development [107]. A significant reduction in the SENP1 expression

has been attributed to the physical disruption of an intron enhancer element; this mutation reduces both deconjugation and processing of SUMO-1. The physiological consequences of the loss of SENP1 function during mouse development are apparent after midgestation in the placenta. These abnormalities result in placental abnormalities incompatible with embryonic development.

6.3. Growth and differentiation

In yeast, Ulp1 is essential for the G_2/M cell cycle transition, but Ulp2 is not essential for cell cycle progression [5,10,36]. Ulp2 mutants show the reduction of chromosome stability, temperature sensitive growth and increased sensitivity to agents that cause DNA damage [9,108]. Recently, it has been reported that Ulp2 is required for resumption of cell division following termination of the DNA damage checkpoint, although it was not required for DNA double-strand breakage repair. When non-repairable DSB was introduced by HO endonuclease, Ulp2 deletion mutants activated and maintained a normal DNA damage checkpoint arrest, and then terminated checkpoint signaling; then a large fraction of these mutants caused arrest at post-metaphase [109]. The Ulp2-H531A catalytic-site mutant demonstrates permanent arrest after DNA damage similar to ulp2 deletion mutant, suggesting that one or more proteins sumoylated following checkpoint activation interfere with the successful completion of mitosis. Notably, the cell growth defects in *Schizosaccharomyces pombe* ulp1 mutants were not rescued by overexpression of the mature form of Pmt3. This suggests that the deconjugating activity of Ulp1 or the desumoylation of some substrates is required for normal cell cycle progression [110].

It has been shown that increased levels of SUMO-1 in synovial fibroblasts (SFs), from patients with RA (arthritis), contribute to the resistance of these cells to Fas-induced apoptosis. This phenotype was conferred by increased sumoylation of nuclear PML, and increased the recruitment of the pro-apoptotic molecule Daxx to PML NBs. SENP1 can reverse the apoptosis-inhibiting effects of SUMO-1 by releasing Daxx from PML-NBs [111]; in addition, it is expressed at a lower level in the RA-SFs. This finding suggests that SENP1 may contribute to the pathogenesis of inflammatory diseases such as RA. Mammalian NAD^+-dependent histone deacetylase SIRT1 deacetylates and inactivates apoptotic proteins such as p53. SIRT1 is a SUMO modification target; SUMOylated SIRT1 is active and suppresses the activity of its apoptotic substrates. SENP1 promotes apoptosis in cells exposed to genotoxic stress by interacting and desumoylating SIRT1 [112]. The critical role of SENP1 in TNFα-induced ASK1-JNK activation and endothelial cell apoptosis is conferred by the translocation of SENP1 from cytoplasm to the nucleus in response to TNF [113]. In the nucleus, SENP1 mediates desumoylation and translocation of HIPK1 to the cytoplasm, leading to enhanced ASK1-JNK dependent apoptosis. Recently, it has been demonstrated that SENP5 is required for mammalian cell division [94,114]. Knockdown of SENP5 by RNAi leads to a dramatic decrease in HeLa cell proliferation and the appearance of many cells with multiple nuclei and aberrant nuclear morphology, in particular a multilobular morphology, indicative of defects in mitosis and cytokinesis.

6.4. Cancer and other diseases

There are some reports on desumoylating enzymes and tumorigenesis. SENP1 has been upregulated over two fold in thyroid oncocytic tumors using a two-step differential RT-PCR assay [115]. In addition, it has been reported that SENP1 is overexpressed in human prostate cancer specimens. Transgenic expression of SENP1 in mouse prostate epithelium leads to the development of early neoplastic lesions in the prostate [116]. However, SENP1 expression has been shown to be reduced in metastatic cancer cell line, LNCaP, compared to RWPE1 normal prostate epithelial cell line [102]; these findings

suggest that the contribution of desumoylating enzymes in cancer development and metastasis appear to be different [117]. Constitutional t(12;15)(q13;q25) translocation between SENP1 and MESDC2 (embryonic polarity-related mesoderm development gene 2) that disrupts both genes was identified from a human patient with infantile sacrococcygeal teratoma [118]. A chimeric SUSP1-T cell lymphoma breakpoint associated target (TCBA) 1 gene, located at chromosome band 6q21, in T-cell lymphoblastic lymphoma cell line HT-1, has been identified [119]. This chimeric mRNA expresses a truncated SUSP1 after nucleotide 550 due to a frameshift at TCBA1 in exon 4. Altered subcellular localization of SENP5 in patients with oral squamous cell carcinoma (OSCC) has been reported. SENP5 predominantly localized to the cytoplasm of OSCC and SENP5 expression was associated with differentiation of OSCC cells [120]. These findings implicate a possible role for SENP family in cancer development.

6.5. RNA biogenesis

There have been several studies demonstrating the effects of sumoylation/desumoylation on proteins potentially involved in RNA metabolism. Ulp1 is genetically linked to the 60S export factor Mtr2, and plays an important role in 60S pre-ribosome particle export [121]. Ulp1 has been localized primarily to the nuclear pore complex (NPC) via its interaction with the two karyopherin nuclear transport factors Kap121 (Pse1) and Kap60–Kap95 [71]. The filamentous proteins Mlp1 and Mlp2 and the nucleoporin Nup60 have also contributed to the localization of Ulp1 [122,123]. Mlp1, Mlp2 and Nup60 are involved in a RNA quality-control pathway that prevents leakage of improperly processed mRNA from the nucleus. Esc1 is a non-NPC nuclear envelope protein that controls the proper assembly of the nuclear basket. Loss of Esc1 results in the retention of unspliced pre-mRNA in the nucleus, and Esc1 modulates Ulp1 localization at the nuclear envelope. Ulp1 is also required for normal nuclear pre-mRNA retention by a mechanism genetically linked to Esc1 and Mlp1 [122]. Thus, it is possible that the strategic localization and the activity of Ulp1, at the NPC, allows for surveillance of mRNA export during nuclear export.

It has been reported that two proteins involved in the pre-mRNA 3′ processing, CPSF-73 and symplekin are sumoylated. SENP2 interacts with these proteins and inhibits 3′ processing in HeLa nuclear extracts *in vitro* [124]. However, whether SENP2 influences 3′ processing *in vivo* requires further investigation. Recently, SENP3 has been shown to be crucial for 32S rRNA processing in the nucleolus [125]. NPM1 is required for the processing of the 32S rRNA species to mature 28S rRNA. SENP3 cleaves SUMO-2 from NPM1 and this process is a critical step for 28S maturation in the nucleolus. The related nucleolar SUMO protease, SENP5, is unable to deconjugate SUMO-2 from NPM1 and does not affect 32S processing. However, knockdown of SENP5 reduces the amount of the primary 47S rRNA transcript, indicating that SENP3 and SENP5 play a role in ribosome biogenesis by acting on distinct substrates [125]. Another group suggested new SENP3 and SENP5 targets for their role in ribosome biogenesis [126]. SENP3 and SENP5 codepletion enhanced the 60S ribosomal subunit RPL37A and 60S pre-ribosomal export factor GNL2 sumoylation. They speculated that NPM1 acts an adaptor for targeting of SENP3 and SENP5 to ribosomal proteins.

6.6. Chromosome maintenance

Yeast Ulp2 is required for the maintenance of chromosome structures. Ulp2Δ mutant strains are impaired in the mitotic-specific targeting of the condensin complex to chromatin, in particular rDNA chromatin [108]. In addition, Ulp2 mutants display an increase in premature separation and fail to maintain cohesion at the regions proximal to centromere, which is caused in part by the poor regulation of the SUMO modification status of DNA Topoisomerase II (Top2)

[127,128]. The role of Ulp2 in cohesion is to control the SUMO modification of proteins involved in organizing centromeric chromatin into a structure that supports the centromere cohesion dynamics. Precocious Dissociation of Sisters gene (Pds5p) is important for sister chromatid cohesion; Ulp2 overexpression efficiently suppresses the temperature sensitivity and precocious sister dissociation in pds5 mutants [128]. Pds5p sumoylation promotes the dissolution of cohesion and Ulp2 activity modulates this sumoylation. Recently, it has been shown that inactivation of Ulp2 weakens condensin association with the kinetochore [129].

6.7. Others

Murine SUSP4, which is closely related to human SENP2, has been found in association with p53 and Mdm2 *in vivo*. SUSP4-mediated desumoylation of Mdm2 promotes self-ubiquitination activity of MDM2 and its destabilization, thereby leading to p53 stabilization [48]. The expression of SUSP4 is induced by UV damage, but not by other DNA-damaging agents, such as etoposide and camptothesin, suggesting that SUSP4 responds to a limited range of stress.

Using SENP1 knockout mice, Cheng et al. demonstrated the important physiological role of SENP1 in the hypoxic response, and identified SENP1 as the specific isopeptidase that removes SUMO from HIF1α [130]. SENP1 knockout embryos show severe fetal anemia caused by reduction in the erythropoietin (Epo) production that results in early embryonic lethality. SENP1 was found to control Epo production by regulating the stability of HIF1α during hypoxia. HIF1α is rapidly degraded in SENP1 knockout mouse embryonic fibroblasts even under hypoxic conditions, whereas restoration of SENP1 markedly enhanced HIF1α activity. Hypoxia-induced sumoylation of HIF1α, which promotes its binding to an ubiquitin ligase, von Hippel–Lindau (VHL) protein, in a proline hydroxylation-independent manner, leads to its ubiquitination and degradation. SENP1 deconjugates sumoylated HIF1α and allows HIF1α to escape from degradation under hypoxic conditions [130].

The role of SENPs has expanded to include the regulation of mitochondrial dynamics [131]. The cytosolic pool of SENP5 plays a role in the regulation of mitochondrial morphology, at least in part, by its effects on the mitochondrial fission GTPase DRP1 desumoylation. Silencing of SENP5 results in a fragmented and altered morphology of mitochondria and significant increases in the production of free radicals, suggesting a role for SENP5 in mitochondrial morphology and metabolism [131].

SENPs have been shown to operate at the plasma membrane controlling ion channel function. The voltage-gated potassium channel Kv1.5 has been identified as a target of SUMO and SENP2 leads to a selective hyperpolarization shift in the voltage dependence of steady-state inactivation [132]. SUMO modification silences the leakage at the K$^+$ channel K2P1 and removal of SUMO by SENP1 overexpression increases the K2P1 channel activity in *Xenopus* oocytes [133].

7. Concluding remarks

The list of characterized desumoylating enzymes appears to be short considering the large number of target proteins that are modified by SUMO. In the ubiquitin system, there are five classes of deubiquitinating enzyme (DUB) families: four are cysteine proteases and only one is metalloprotease family. The human genome encodes approximately 100 putative DUBs [134]. In yeast, at least 17 DUBs have been identified by virtue of the conserved catalytic domains. Although they have been identified over several decades, new DUBs continue to be reported and it is unclear how many desumoylating enzymes remain to be identified. Even though the substrate specificity of Ulp/SENPs is dictated by the subcellular localization of each protease, there is very specific substrate recognition in some cases. The nonconserved

N-terminal regions of the Ulp/SENPs can provide limited and selected substrate specificity by targeting the protease to unique subcellular compartments or the direct recognition of substrates. To get more important information about the substrate recognitions by Ulp/SENPs, information of crystal structures of them will be helpful. Although many SUMO targets are in the nucleus, it is clear that sumoylation takes place in all areas of the cell. Some SENPs can be found in areas that are not their main territory; however, SENPs are mainly located in the nucleus-associated organelles. How are the proteins sumoylated outside of the nucleus regulated by desumoylating enzymes? Are they regulated by the shuttling of SENPs in the cell? How the shuttling is regulated? The modification of these enzymes or the specific binding partners might be important to the regulation of these enzymes; or perhaps new cytosolic desumoylating enzymes might be active in different locations. The regulatory mechanisms of Ulp/SENPs also require further elucidation, and the attempt to answer these questions will be the subject of future investigations.

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